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STUDIES ON THE POLAROGRAPHIC SERUM REACTION

BY

STURE ÖSTERLIND

STOCKHOLM

1955

UNIVERSITY OF MICHIGAN

To my wife

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PREFACE

This study on the polarographic serum reaction has been in progress since 1945. On account of several reasons the work has been considerably delayed.

The experiences in this and adjacent subjects that have been made since 1945 have, however, according to the author's opinion, rather increased the current interest for these problems.

I want to express my most sincere thanks to Dr Birger Drake at the Biochemical Institution, University of Upsala, for his invaluable advice in biochemical problems and to Dr Erik Blomgren at the Chemical Institution, University of Upsala, for his kind interest in my work.

For advice and help with the mathematical and statistical work I am gratefully indebted to Dr Arne Sollberger, Stockholm.

My wife assisted me all the time with the laboratory work and never tired of spurring me on with advice and encouragement.

Stockholm, July, 1952.

Sture Österlind

PART I

Historical Review and Plan for the Author's Investigation

INTRODUCTION

Investigations carried out by Waldschmidt-Leitz and his co-workers as well as others (124, 150, 158) have shown that serum from healthy subjects possesses greater power to reactivate inactivated papain and cathepsin than sera from cancer patients. This effect was attributed to the sulphydryl groups in serum. Rudolph Brdicka, who had already previously studied the polarographic conduct of the disulphidic and sulphydryl groups, took up the question afresh, and in 1936 his studies led to the presentation of the so-called "Polarographic Cancer Reaction."

Continued investigations very soon confirmed that here was a reaction that was "positive" in the majority of cancer cases, though—as might be expected—it also proved positive in a number of other diseases, primarily those connected with fever. In addition the reaction proved negative in a small number of cases with cancerous tumours.

Despite the non-specificity for cancer, which was clearly shown by the reaction from the very first, most scientists that have studied it have considered it to be a valuable help when diagnosing cancer and also an objective barometer of the malignity, degree of development and course of both this as well as certain other diseases.

The writer has considered it possible that the reaction might give material for other viewpoints concerning protein disturbances in cases of malignant tumours and other diseases involving protein disintegration. Nevertheless the writer wishes to point out from the

first that the reaction must be regarded as an expression of the appearance of proteins having undergone a pathological change and decomposition products of proteins in serum in quite general terms. The writer has therefore chosen to give the reaction the name of *"The Polarographic Serum Reaction."*

Literature, however, presents very contradictory results, and statements available concerning the standardization of the method are incomplete. Moreover, the question of the true nature of the active substances has not yet been investigated. In this work, therefore, the writer has endeavoured to present a further explanation of the conditions which can enable a routine analysis to be carried out with a satisfactory reproducibility, and he has also attempted to contribute to the question of the nature of the substances producing the reaction.

The writer has based his work on the statements available in literature concerning the Waldschmidt-Leitz alcohol fractionating method. A clinical test was carried out on a considerable number of sera from patients with a diversity of diseases parallel with the methodological investigation. Continued studies on the method, however, have resulted in indications of further improvements in the method. The results of the clinical tests in order to prove the practical value of the method will therefore not be published until the questions concerning the method have been solved.

CHAPTER 1

Characteristics of the Polarographic Analytical Method

The principle on which the polarographic analytical method is based can be summarized as follows: If a solution of substances which are electrochemically reducible or oxidizable (dissociated or non-dissociated) are electrolyzed at a continuously increasing voltage and with a dropping mercury electrode as a cathode (when analysing positively charged particles) or an anode (when analysing negatively charged particles), then, when the reduction or oxidation of the substances in question takes place, such changes are obtained in the current-voltage curve produced as make both qualitative and quantitative determination possible (5, 6, 17, 18, 38, 63, 64, 66, 111, 120, 164).

The first step in the development towards polarography was taken by G. Lippmann in 1873 with the construction of the so-called capillary electrometer which consisted of a fine glass capillary filled with mercury. The object was to study the movement of the mercury in the capillary when changes were taking place in the surface tension owing to the mercury beginning to get polarized. In these experiments a large mercury surface consisting of a bottom layer in the electrolysis cell served as the opposite electrode. Certain physical and chemical changes take place while the current is passing through the minimal mercury surface in the capillary, and these changes are described as "polarization." The larger mercury electrode, on the other hand, will be physically and chemically unchanged as a matter of fact, i.e. it can be regarded as unpolarizable on account of the considerably smaller current density on its much larger surface.

In 1903 G. Kucera modified the Lippmann method by increasing the pressure of the mercury in the capillary so that it dropped

slowly off the tip instead. By weighing the drops of mercury Kucera could study the variations of the surface tension at the polarization. Kucera recorded the values obtained in a so-called electro-capillary curve (fig. 1).

In certain cases, particularly when he used strongly diluted electrolyte solutions, the curves showed peculiar "anomalies", which he termed "secondary maxima." In 1918 Kucera's pupil, J. Heyrovsky, began to study these "anomalies" in the electro-capillary curves, and then he found that the electrochemical processes on the drop electrode could be better studied by measuring the strength of the current than by determining the surface tension. Heyrovsky introduced a sensitive galvanometer into Kucera's apparatus, by means of which he was able to measure the variations arising in the strength of the current. In 1925 with M. Shikata he constructed an instrument for the automatic recording of the curves, and this was termed a polarograph. The current voltage curves recorded were called polarograms.

The advantages with the dropping mercury electrode in polarographic analysis chiefly consist in it being possible to make the surface of the electrode very small and consequently extremely polarizable, at the same time as the current factors—intensity of current, voltage and resistance—can be accurately reproduced thanks to the regular dropping. Constantly fresh mercury surfaces make their appearance during the dropping, and they are independent of the polarizing processes that took place on the preceding drops. This means that the strength of the current is dependent only on the voltage applied, but independent of the time for the electrolysis. There is no noticeable change in the concentration of the solution, since the quantity of the substance precipitated on the drops is usually infinitesimally small as compared with the total quantity in the solution. The analyses can therefore be repeated an almost unlimited number of times on one and the same sample, without there being any noticeable reduction in its concentration. The considerably high overvoltage of the hydrogen at the mercury surface also makes it possible to reach extremely negative potentials without development of hydrogen gas. Finally the little capillary, thanks to its being so small, makes it possible to analyse extremely small quantities of fluids (down to 0.005 ml.).

The very simplest "polarograph" is shown schematically in fig. 2. The electrolysis cell (A) contains the sample to be analysed. The electrodes are connected with the measuring instrument (the polaro-

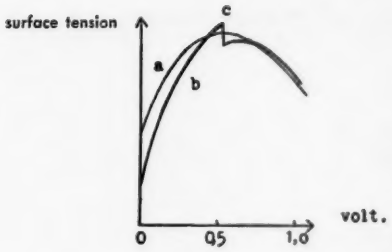

Fig. 1. Electrocapillary curves.
a = Lippman's curve. b = Kucera's curve. c = "Secondary maximum" in Kucera's curve.

graph) so that the dropping mercury electrode forms the cathode (E) and the layer of mercury at the bottom of the vessel is the anode. The cell is connected to an accumulator (B) through the electrodes, the former providing the electromotive force required for the electrolysis. A sensitive galvanometer (G) is connected into the current circuit as well as a variable resistance (R), which allows of a continuous change of voltage.

The galvanometer can be optically connected to a camera with rotating film, or the galvanometer can be replaced by a special amplifier, which is connected to a stripchart recorder.

In the system of co-ordinates, in which the current-voltage curve —the polarogram—is recorded, the electromotive force is obtained on the abscissa and the strength of the current on the ordinate. The polarogram recorded is distinctive on account of the variations in the strength of the current, which are conditioned by the reduction or precipitation potential given for each substance. As far as the metals are concerned, these potentials broadly speaking correspond to the electrolytic voltage chain (fig. 3). Reference should be made to original literature for the mathematical deduction of the curves (68, 70, 77, 121).

The little "jags" forming the curves (fig. 3) are due to the dropping of the mercury. The falling part of each "jag" is due to a decrease in the strength of the current as the polarization proceeds on the drop, while the rising part reflects the genesis of a fresh mercury surface after the fall of the previous drop.

At this point it is necessary to give a brief account of the

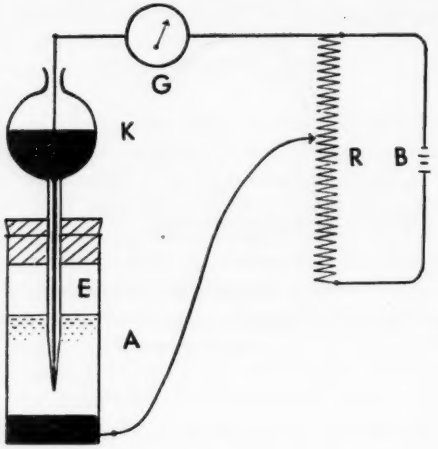

Fig. 2. The principle of the construction of the polarograph.

mechanism leading to the origin of the curves in order to understand the basis for the quantitative and qualitative measurement of the polarograms.

As an example, let us analyse the percentage of cobalt in a solution containing cobaltous chloride. If the electromotive force in the system is increased from 0 volt, a current-voltage curve will at first be obtained that is practically parallel to the abscissa (fig. 4). This is due to the externally applied and the internal voltages which originate in the limit surface between the electrolyte and the electrode balancing each other. When the outer voltage finally reaches the reduction protential of the cobaltous ions, there is a precipitation of the cobaltous ions on the surface of the mercury drop. Thanks to the liberation of the quantity of electricity, there is an increase in the strength of the current, which is recorded in the polarograph as a steep increase in the curve. The strength of the current increases exponentially with the voltage.

The exponential increase in the curve is, however, shortly afterwards interrupted owing to the increased precipitation on the surface of the cathode, causing the electric double layer to be exhausted of cobaltous ions. At this moment the strength of the current is no longer dependent solely on the polarization phenomenon, but its

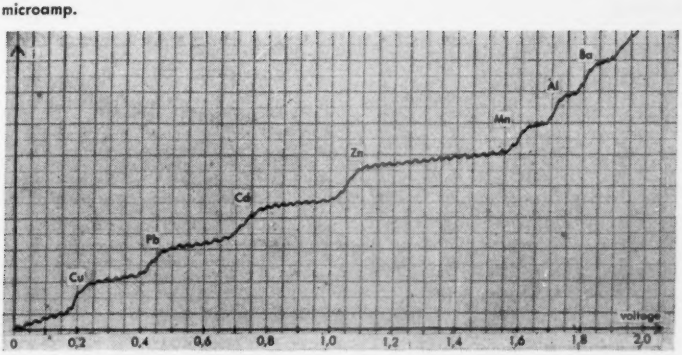

Fig. 3. A polarographic "spectrum."

magnitude is determined by the diffusion of frest cobaltous ions to the double layer. Only as many cobaltous ions can now be discharged per time-unit as enter the double layer by diffusion. Thus, the continued course of the curve corresponds to the diffusion current. Between this and the ion concentration in the solution there is direct proportionality within certain limits, and therefore the height of the "step" or the "wave" formed is directly proportional to the ion concentration of the solution.

If there are several cations present in the solution, the diffusion current will, when the next reduction potential is reached, again resume the form of an exponential curve, which will in its turn become a diffusion current curve, etc. The height of each step formed is proportional to the concentration of the respective ions in the solution. In this way it is possible to determine a number of various substances simultaneously—in some cases up to as many as 6 or 7.

The polarogram obtained can be used for both qualitative and quantitative analyses. In this work there are only quantitative measurements, and consequently they are the only ones described in detail.

The quantitative measurement is based on a measurement of the diffusion current. It must then be remembered, however, that all the affecting factors must be kept konstant. These factors are contained in Ilkovic's formula

$$i_d = 605 \cdot n \cdot D^{1/2} \cdot C \cdot m^{2/3} \cdot t^{1/6}$$

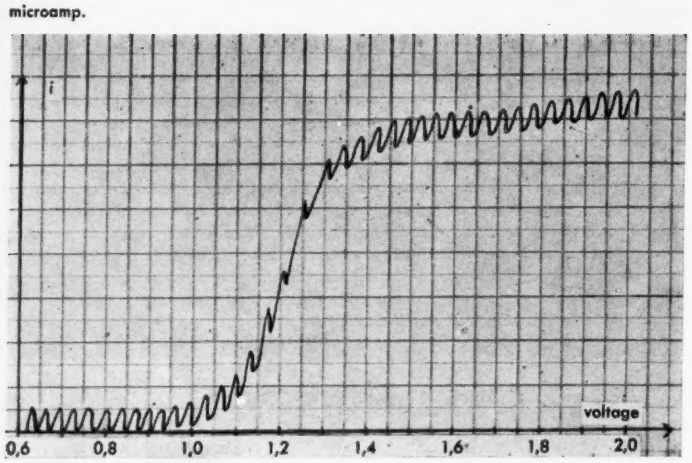

Fig. 4. Typical polarogram, obtained when analysing a solution containing cobaltous chloride.

where i_d is the diffusion current in microamperes, n the number of faradays of electricity required per mole of substance taking part in the electrode reaction, D the diffusion coefficient of the reducible or oxidizable substance in the unit cm^2 $sec.^{-1}$, C its concentration in millimoles per liter, m the rate of flow of mercury from the dropping electrode capillary expressed in the unit mg. $sec.^{-1}$ and t the drop time in seconds.

The formula shows that the analyses must be carried out at a constant temperature, since, according to Ilkovic's calculations with every degree in the change of temperature (about normal room temperature), the diffusion current changes by about 1,6 %. In addition the speed at which the mercury runs out and the drop time must be kept constant.

The measurements of the diffusion current can be made in various ways. Provided that the same method is always used for one and the same investigation, it is immaterial which method is chosen. The method adopted in this work is described in greater detail in chapter 7.

For further information concerning the polarographic method reference should be made to nos. 7, 69, 76, 89, 90, 134, 137 and 141 in the references.

CHAPTER 2

The Polarographic Protein Reaction

If blood-serum is mixed with a solution containing cobaltihexammine chloride in ammonia, buffered with ammonium chloride, when the solution is polarographically examined there will be a so-called "double wave", the height of which varies according to the quantity of serum added (13). The wave is not to be found when serum alone is polarographically examined, there must also be present in the first place cobalt, though it may also appear with some other metals, e. g. nickel. If serum is polarographically examined in indifferent electrolytes, e. g., ammonium chloride, another "protein wave" will be found near the reduction potential of the alkali ions (8, 14). In the results obtained with ammonium chloride, when it was established that the height of the wave depended on the concentration of the ammonium ions, it was concluded that the proteins catalytically diminished the overvoltage of the hydrogen ions at the electrode, and the consequence is that they are deposited at a more positive potential than when serum proteins are not present. In solutions containing cobalt the serum proteins show a similar catalytical effect, in consequence of which hydrogen is deposited at the electrode.

An investigation of the individual amino acids of which the proteins consist showed that the effect was only produced by cysteine and cystine (19, 31) and that proteins not containing these amino acids did not evoke any polarographic wave. The catalytic nature of the process could be proved by the cystine wave being able to rise to values that were hundreds of times higher than those which correspond to a simple reduction of the cystine.

Brdicka (19, 31) also showed that the catalytic effect must in the first place be ascribed to the sulphydryl groups, since he found

that the cysteine and the cystine waves were equal in size, when the molar concentration of the first mentioned amino acid was double the latter (39). Brdicka also showed that nearest the electrode at the potential at which the catalytic process takes place the disulphidic groups are completely reduced, and this explains this relation.

The part that the sulphydryl groups play in the reaction can also be tested by the monoiodoacetate reaction (72), in which case the iodoacetate ion in alkaline solution reacts with the sulphydryl groups according to the following formula:

$$-SH + JCH_2COO' \rightleftarrows -SCH_2COO' + H\bar{J}$$

The iodoacetate ions, on the other hand, remain indifferent to the disulphidic groups. The cysteine, when affected by the iodoacetate, may therefore be expected to lose its polarographic effect, whereas the cystine would retain its activity, the rapidity of the reaction not being quick enough to have time to attack the freshly formed sulphhydryl groups, which arise from the electro-reduction of the disulphidic groups. Such is the case, too, as Brdicka inter alia has shown (19, 31, 39). By this means it is possible to distinguish between cystine and cysteine polarographically by comparing the polarographic curves before and after addition of monoiodoacetate.

There is, however, a difference between the polarographic curves produced by the amino acids and proteins, the latter giving a reaction with both divalent and trivalent cobalt, while the catalytic cysteine wave only appears in the presence of divalent cobalt. This makes a genuine protein effect possible when there is a simultaneous presence of the amino acids or low molecular polypeptides with cysteine or cystine in the molecule. If equimolar solutions of ammonia and ammonium chloride are used (0·1 N), the protein will show a typical double wave, whereas free cysteine or cystine shows a single wave. Since the double wave with protein is transformed into a single one identical with the second wave in the double wave (the cystine wave) when an excess of ammonia is added, Brdicka is of the opinion that the way in which the cysteine or the cystine is bound in the molecule causes the first wave in the double wave (144, 146).

The height of the protein wave increases with increasing protein concentration. The increase, however, is not linear. Instead there is a tendency to reach an upper limit (13, 19, 31, 35, 39). Brdicka has

pointed out that a mathematical analysis of the curve shows that its course corresponds to Langmuir's equation for an adsorption isotherm (35, 39). The formula for this is

$$c = \frac{ab[\text{Prot}]}{1 + b[\text{Prot}]}$$

in which [Prot] signifies the concentration of protein in the solution, c the concentration of the protein adsorbed on the mercury drop and a and b empirical constants. The height of the protein wave is proportional to the concentration of protein on the mercury drop and further to the number of polarographically active groups in the protein. As will be seen from Langmuir's theoretically derived formula, the height is, however, not directly proportional to the concentration of protein in serum.

The cobalt ions exercise a twofold influence on the height of the polarographic curve. Firstly the curve assumes a step, which is entirely due to the cobalt, the height of which is constant and can simply be subtracted from the total height. Secondly the rest of the height, which is caused by the protein, depends, simultaneously on the concentration of the cobalt ions. This relation is expressed by the formula

$$H = [\text{Co}]^{0.74} \cdot \frac{ab[\text{Prot}]}{1 + b[\text{Prot}]}$$

in which H signifies the height of the protein wave. In routine work, however, the cobalt concentration is kept constant. Thus, in calculating the protein concentration a simplified formula may be used:

$$H = \frac{a_1[\text{Prot}]}{1 + b[\text{Prot}]}$$

Moreover, the height of the protein wave depends also on pH. Hence, when analysing, buffered solutions must be used, the most suitable being equimolar solutions of ammonia and ammonium chloride (0·1 N).

A matter of interest for the serum reaction is that in determining cystine or cysteine the height of the curve is affected by other amino acids, particularly histidine, arginine, tryptophane and phenyl-

alanine. As long as the protein has a sufficiently high cystine content as compared to the amino acids mentioned, the analysis can be carried out all the same without any noteworthy disturbance. Satisfactory analyses can be directly carried out on albumin and globulin fractions in human blood serum (15). Finally it must here be mentioned that glutathione, cysteylglycine, thioglycollic acid and possibly even other thio-acids give a polarographic catalytic wave of a similar character (14).

Thus, the polarographic protein reaction is produced by all proteins containing cysteine or cystine. In quantitative measurements it must also be noticed, however, that the protein molecule undergoes structural changes thanks to the effects of different agents. It is known that some of the disulphidic and the sulphydryl groups are "camouflaged" in the protein molecule and therefore all the sulphuric groups in the protein molecule cannot be expected to take part in the polarographic reaction. On the contrary, their groups are very likely not active polarographically, except when they are "liberated." The simultaneous polarographic and colorimetric investigation of protein treated with alkali or ultraviolet rays (19, 31, 72) has shown that the double wave increases simultaneously with the colorimetric increase of the sulphuric groups under the denaturation process. Moreover, Brdicka and others have shown that denaturation not only involves an increase in polarographic activity, but it also causes changes in the shapes of the curves. A similar effect is also brought about by proteolytic enzymes (40). In these cases there is a considerably greater increase in the protein wave than in the denaturation tests, and the polarographic waves can rise to values which can only be compared with pure cystine. Brdicka (40) says: "It seems therefore that the limitation of the maximum height of the catalytic wave is connected at the same time with the size of the respective molecules, the participation of all active groups in the catalytic electrode process being thus spatially hindered. Indeed, the smaller the polypeptide resulting from the proteolytic or hydrolytic protein degradation, the greater is the maximum height of this catalytic wave."

The discovery mentioned in the introduction, that inactivated papain and cathepsin were activated in varying degrees by serum from healthy subjects and cancer patients was attributed to the effect of the sulphydryl groups (15, 26, 152). Brdicka showed that with native serum it was possible to obtain a difference polarographically, though it was slight. If on the other hand the serum was

denaturated by means of alkali, pepsin-hydrochloric acid, heat etc. previous to the analysis, the difference was found to be much more pronounced (19, 27, 28, 29, 31). Brdicka showed by hydrolysis tests that sera from cancer patients contained smaller quantities of cystine than sera from healthy subjects (27, 34).

Detailed studies on the protein reaction have also been carried out by Tropp and his co-workers (144—148).

CHAPTER 3

Brief Survey of Earlier Work on the Polarographic Serum Reaction

Work within the sphere of serodiagnostics has led to the discovery of certain general pathological changes in blood serum in certain diseases. It is true that these changes are not specific for the disease in question, though they are nevertheless of considerable value when it comes to recognizing it. In order to understand the polarographic serum reaction it will be necessary to give a brief survey of earlier serodiagnostic work that has led to this reaction.

The direct separation and appreciation of the various serum fractions in normal and pathological serum must be regarded as being the forerunner of this reaction (74, 162). Svedberg and his coworkers (138) carried forward these classifications of the serum protein by making use of the ultracentrifuge, thus making it possible to characterize protein fractions in serum by determining their molecular weights.

Thanks to Tiselius' electrophoretic method (139, 140) it has been possible to distinguish other fractions in serum with different electrochemical qualities though with about the same molecular weight. The old classification of the plasma protein in albumin, globulin and fibrinogen was confirmed by these investigations, at the same time as it was shown that the serum globulin consists of several protein components.

Different diseases show in plasma an increase or a decrease in normally occurring protein fractions or pathological proteins make their appearance. The most familiar changes in the first category are hyperfibrinogenemia and hyperglobulinemia during infections and diseases with high temperatures: also in cases of malignant

tumours, active tuberculosis etc. These changes may also be associated with an increased sedimentation reaction of the red blood cells.

A decrease in the protein fractions also characterizes certain pathological states. The confirmation of similar changes between the various serum fractions is of a limited value for diagnosis, though they can nevertheless be made use of when judging the actual status of the pathological process. From a diagnostic viewpoint, however, it is more important to study the qualitative changes that may occur in serum and to confirm the presence of pathological protein.

The direct proof that pathological protein fractions exist in serum in the case of certain diseases has been given by Macfarlane (83—86). In a case of a tumour in the wall of the gall bladder with obstructive icterus he found by means of ultracentrifuging not only the three normal fractions (A, X, G) but also a fourth having a lower molecular weight than albumin. He indicated the new fraction by the sign A_1. Macfarlane has also found another pathological protein in myeloma. Its molecular weights is between X and G, and he has called it Y.

The polarographic method provides yet another possibility for the study of changes in the serum protein as well as the appearance of fresh pathological fractions, provided that they contain disulphidic or sulphydryl groups in the molecule (13, 14, 16, 23—30, 33, 34, 39, 41, 57, 58, 60, 142, 143, 153). According to what has been said in chapter 2, however, it is impossible to form a direct distinction between the two groups.

The origin of the polarographic protein reaction can in its turn be traced back to Waldschmidt-Leitz and his co-workers' enzymatic studies on the coagulation conditions of the blood in cases of malignant tumours (124, 150—155, 158).

The existence of a natural activator for the catheptic enzymes had been shown and studied by several scientists, inter alia Waldschmidt-Leitz (150) and Schäffner (136). Waldschmidt-Leitz (158) succeeded with Purr and Balls in obtaining this activator in a crystallized form and was thus able to identify it as glutathione. The manner of its effect was studied by inter alia Grassman, Dyckerhoff and Schoenebeck (61) and also by Kühnau and Morgenstern (80, 81), who showed that the glutathione at a considerably lower concentration than cysteine neutralizes the power of the blood to coagulate, and that substances blocking the SH groups, e.g. iodoacetate, neutralize the effect of the glutathione when mixed in suitable concentrations.

Purr and Russell (124) could prove that the effect of papain and cathepsin is allied to the SH groups and that these latter will alone be responsible for the increased activation in the enzymes mentioned. They also showed that cancer blood has a less activating power on papain and cathepsin than normal blood has.

Finally Purr felt that he could contend (122, 123) that the actual activator for papain and cathepsin is solely the SH groups and that the two enzymes in an active state are proteins containing SH groups. Further studies on these conditions were carried out by inter alia Waldschmidt-Leitz, Bergman, Abderhalden, Köhler (1, 11, 82, 110, 151, 152, 154, 155, 157).

In 1933 the Czech scientist, R. Brdicka, began to study the SH groups in serum by means of the polarographic method (13—16, 23—26). His investigations resulted in a long series of publications, in which the method was tested and developed still further (19—22, 32, 36, 37).

When a direct analysis was made of serum in a suitable dilution with buffered cobalt solution, the decrease that was expected in the SH groups in cancer sera was observed, though it was fairly slight. By denaturating serum with e.g. KOH, a considerable increase was, however, obtained in the active groups with a more pronounced difference between normal and pathological serum. The same result was obtained by treating serum with pepsin-hydrochloric acid (27, 28).

From the very first, however, Brdicka realized that the reaction was not specific for cancer, as the same differences also arose in infectious and inflammatory diseases. Nevertheless Brdicka pointed out—as several other scientists did subsequently—the practical importance of the increase in the strength of the reaction the larger the tumour became. By carrying out a polarographic and colorimetric analysis of the cystine percentage in the hydrolysate of serum Brdicka clearly showed (30, 33, 34) that a lower polarographic double wave meant a smaller quantity of active groups liberated during the denaturation.

Tropp (142, 143) observed quite early that the strength of the reaction also depended on the total quantity of proteins in serum. In order to eliminate this error Tropp introduced a simultaneous refractometric protein determination of serum, which thus enabled him to correct the polarographic curves obtained and bring them to a uniform level. In this way he managed to eliminate several cases of misleading results in consequence of variations in the

protein content of the serum. Tropp's assertion that the sedimentation reaction for the red blood cells ran parallel with the polarographic serum reaction has since been shown not to be perfectly valid by inter alia Wedemeyer, Daur, and Regamey (125, 160).

Brdicka's original reaction was, however, very soon followed by the so-called filtrate reaction, which in principle implies that after denaturation the high molecular proteins in serum are precipitated with e.g. sulphosalicylic acid or alcohol. The filtrate is then found to have more sulphydryl-containing constituents in cancer than in the case of normal sera, thus the contrary of the original reaction. The differences between normal and pathological sera are also considerably more pronounced than is the case in the original reaction. The new reaction became the object of industrious study especially by Brdicka (29, 33), Novak and Klumpar (41) and also Waldschmidt-Leitz (153). A number of other scientists tested the method clinically, and broadly speaking their results were in agreement (3, 9, 10, 12, 49, 50, 54, 87, 88, 101—104, 135, 159). The most usual method adopted seems to be denaturation with KOH and subsequently precipitation of the proteins with sulphosalicylic acid.

According to the majority of scientists in this field the filtrate reaction is caused by decomposition products for the serum protein, which are liberated at the denaturation and are soluble in sulphosalicylic acid or alcohol of a certain concentration.

Brdicka showed that the active substances cannot be dialyzed through parchment or cellophane membrane. They give polarographic reaction with both divalent and trivalent cobalt, and therefore it must be presumed that the weight of the molecule is comparatively great (34, 41). They are of an albumose or peptone character and contain cystine, which has been proved by hydrolysis investigations with a colorimetric determination of cystine. It was also proved that the relation between heights of the filtrate waves before and after the hydrolysis was maintained.

By treating normal serum with pepsin a polarographic filtrate wave is obtained after the precipitation of the protein, which wave reaches as high values as those of the filtrate wave in pathological sera.

Parenteral supply of foreign proteins produces a rise in the polarographic filtrate wave after only a few hours, and this wave soon reaches a maximum, after which it sinks to a normal level. Foreign proteins in the blood, caused by the decomposition of tumour tissue, the distribution of foreign proteins from infection centres etc. would

cause a decomposition of the serum proteins, and this would be an expression of a "protective reaction of the organism in order to destroy foreign protein". In this process the SH groups would be active. It has not been possible to decide, however, whether these products appear free in the serum or whether they are bound to the serum protein and are only liberated by external manipulations (by the denaturation). It is also possible that these fractions producing the filtrate reaction are directly associated with the pathological protein fractions, which Macfarlane found in serum. Nor is it yet known whether these products constitute a monodispersed or polydispersed system, and so far the attempts to fractionate with various precipitants in order to find a possibility for distinguishing infectious diseases from cancer have not led to any results.

According to Brdicka's opinion everything indicates that the substances causing the filtrate wave are decomposition products of serum proteins of albumose or peptone character and contain cystine nuclei. As a proof of this theory Brdicka refers to tests showing that in pathological sera the disulphidic groups of the proteins decrease, while the decomposition products increase simultaneously.

The hitherto most commonly adopted filtrate reaction with alkaline denaturation and precipitation with sulphosalicylic acid has been practically tested in 20,000 cases by Brdicka, Fort, Ott and Voriskova (55—58, 60). A number of scientists over and above those previously mentioned have tested the method and have generally speaking come to the same result, both practically and with regard to the nature of the reaction, which has been brifly described (45, 67, 71, 73, 75, 79, 91, 93, 94, 97—100, 112—114, 116, 119, 128, 130, 133, 149, 163, 165).

The method has been tested experimentally in various connections. Some tests have been carried out with determinations of serum from rats, mice and rabbits with cancer (42—44, 65, 129, 161), and also with experimental pneumonia on dog (46—48). The results are in agreement with Brdicka's opinions concerning the nature of the reaction.

Investigations have also been carried out to try the use of the reaction for studies of Abderhalden's reaction (2, 46, 78, 117), and in addition comparisons have been made between the polarographic reaction and the non-protein nitrogen. The results are of little interest in this connection (59, 95, 96, 126, 127).

E. Waldschmidt-Leitz and K. Mayer (153, 156), however, tried

to find another way—the one the writer has endeavoured to study more closely. These writers denaturated pathological sera by means of heat and then precipitated with sulphosalicylic acid, which was then removed by dialysis, and the remainder was fractionated with alcohol. When precipitating up to 66% alcohol, a substance was obtained showing a mucoid reaction and containing a small quantity of sulphur. Mayer also considered that the isoelectric point of this mucoid was at pH=3,4 and thus closely corresponded to Blix' submaxillary mucin (12 a). Mayer presumed that the fraction obtained consisted of a mixture of various serum mucoids polluted with small quantities of low molecular proteins. The fraction was presumed to be the cause of the increase in the height of the filtrate wave, which occurred when cancer sera were analysed. In cases of infectious and inflammatory diseases, the writers found that the increase in the height of the filtrate wave was caused by an increase in the content of substances rich in disulphide and sulphydryl groups, which substances could be isolated by fractionating with 66—80% alcohol.

On the basis of these observations Waldschmidt-Leitz and Mayer worked out a filtrate reaction, in which one portion of serum was precipitated with sulphosalicylic acid and another with 66% alcohol. A comparison was made between the respective filtrate curves thus obtained. Several other writers tested the Waldschmidt-Leitz-method, inter alia Felkel (54), Griessmann, Köhler and Söhnel (62), Schmidt (132), Albers (4). They could not, however, observe any definite advantage with regard to the reliability when diagnosing cancer.

Brdicka does not think that this mucoid, which has been found in serum by K. Mayer (92) and others, has anything to do with the polarographic serum reaction. He stands by his opinion that the reaction is produced by decomposition products of the serum proteins, which are of an albumose character. He maintains that this is more likely, as it has already previously been shown that in diseases with a disintegration of tissue, e.g. malignant tumours, pneumonia etc., albumosemia makes its appearance (74, 162). Macfarlane's investigations also confirm these hypotheses to a certain extent, as in similar diseases there is an abnormal spreading of the fractions which move slowly in the ultracentrifugal field, which is a proof of their polydispersity and thus also of their character of decomposition products.

There is a scarcity of data in the literature concerning the relation of the polarographic serum reaction to various physiological

conditions. Podrouzek and Krksoskova (118) have carried out an investigation of the conditions at menstruation, and strangely enough they have found no changes in the reaction during the menstruation cycle. On the other hand (54) Felkel found a premenstrual decrease in the curves, but he carried out no systematic investigations. Oldrich (115) has investigated the reaction during pregnancy as well as before and after birth. During pregnancy he found no changes in the reaction with the exception of a very small decrease in the values towards the end of the time. During the first few days after birth there was a rise in the values, the maximum being reached on the seventh day. On the tenth day they began to sink again, to return finally to the normal values. Oldrich considers the rise to be due to the lactiferous glands beginning to function, which he compares to an inflammatory state.

On account of considerable delay in the publication of the work, the survey of the literature is not complete. A review of the literature published after 1947 will be found in Appendix II, p. 114.

CHAPTER 4

The Purpose of the Investigation

Since Rudolph Brdicka published his original "polarographic cancer reaction" in 1936, several modifications have been worked out. Nowadays, however, it is only the so-called filtrate reactions that are used, and they all have one thing in common, viz. that after the denaturation of serum protein by means of heat, KOH, pepsin-hydrochloric acid etc., the serum protein is precipitated with sulphosalicylic acid or alcohol and a polarographic analysis is carried out on the filtrate. In this way more pronounced differences are obtained between normal sera on the one hand and pathological on the other than is the case with the original, direct method, in which serum protein is not precipitated.

A number of different modifications have been worked out among the filtrate reactions, but taken on the whole they give the same clinical result. Brdicka goes in for denaturation with KOH, after which he precipitates with 20 % sulphosalicylic acid. Waldschmidt-Leitz and Karl Mayer denature the serum by heating it in a boiling water bath, and then it is precipitated with 25 % sulphosalicylic acid. According to a method adopted by the latter writers precipitation is also carried out with alcohol in a second serum portion.

Brdicka measures the height of the double waves obtained and indicates a positive reaction in the cases when the height of the curve exceeds the upper limit of "the normal region." With the same object in view Waldschmidt-Leitz has made use of a comparison between the curves obtained with filtrates after precipitation with sulphosalicylic acid and alcohol respectively. A positive reaction is given in cases in which a decrease in the difference is obtained by subtracting the sulphosalicylic filtrate from that of the alcohol,

which difference is compared with that of the normal value. Waldschmidt-Leitz based his reaction on the opinion previously referred to, that by means of alcohol it was possible to isolate from cancer sera a substance belonging to the mucoid group, which caused the increased polarographic activity in cancer, while in the case of infectious and inflammatory conditions this increased activity was believed to originate from a fraction containing disulphide and sulphydryl groups and with other precipitation properties with alcohol.

Preliminary tests with the Waldschmidt-Leitz method gave certain interesting results, and the writer thinks it probable that further valuable results could be gained here with regard to the nature of the reaction and the possibilities of further development.

In order to achieve more uniform results than have been obtained in the majority of earlier work, the writer considers it necessary to devote extreme care to the purely technical conditions for the reaction in order to obtain fully reproducible values and consequently comparable results from time to time.

Before tests could be carried out with a view to endeavour to fractionate the active substances and to study the physiological variations of these substances, the writer has considered it necessary to carefully study the various factors influencing the analytical result, i.e. the height of the double wave, originating from the different manipulations during the treatment of the serum up to the time of the polarographic analysis. This is necessary, firstly because earlier writers do not seem to have carried out any consistent test in this respect, and secondly because the products causing the reaction seem to be extremely labile under certain conditions, and many factors of a chemical and physical nature may contribute to the occurrence of considerable variations in the final result unless suitable steps are taken for standardization.

This, like the investigation which will be mentioned later — if it is to be considered comprehensive — must include a considerable number of various groups of diseases, since it may be supposed that the carriers of the active groups may vary in composition and thus also as regards their physical and chemical properties. It has, however, not been possible to carry out a comprehensive investigation on account of the extensiveness of the material in comparison with the resources at the disposal of the writer. It has therefore been necessary to limit the investigation of the method to comprising normal sera

as well as some groups of diseases that seemed most elucidating to the writer.

After this the writer has studied more closely the two substances with polarographic activity that Waldschmidt-Leitz states occur in cancer sera and in infectious sera, respectively. With the resources at his disposal the writer has studied the precipitation conditions of these substances as well as their occurrence in normal, cancerous and infectious sera.

An investigation is also carried out concerning the behaviour of the reaction in certain physiological processes, e.g., menstruation, pregnancy, the absorption of foodstuffs, and physical work. The choice has been made so as to make it possible to illustrate the factors that may be of importance also in the practical use of the reaction.

Own Investigations

PART II

CHAPTER 5

Apparatus

The polarographic analyses in this work have been carried out with polarographs manufactured by Radiometer, Copenhagen, the model being PO3.

This polarograph, an illustration of which will be seen in fig. 5, draws the curves straight on to paper by means of a recording pen. The variations in the intensity of current arising during the analysis are amplified by an electronic amplifier, after which they affect a registration arrangement, which records the current voltage curve in ink on the moving sheet of paper. By using an automatically recording apparatus it has been possible to carry out a greater number of analyses and a satisfactory number of controls more quickly than would have been the case with the photographic type.

Moreover, the type used also has the advantage of not being particularly sensitive to knocks and vibrations, and consequently this has facilitated the work. In this respect it has proved quite satisfactory to place the polarograph on a steady table with the three supports resting on 2 cm. thick felt pads.

The potential range of the polarograph lies between —1 and +3 volts with the possibility of adjusting it to —1.5 and +4.5 volts. The highest sensitivity of the apparatus is 1 mm. deflection at a current of 5×10^{-9} amperes. The sensitivity can be varied from the highest figure down to 1/10000 of this value.

Fig. 5. The polarograph.

The movement of the paper is synchronized with the motor-driven potentiometer, the potential range of which is 2 volts. The width of the paper is 10 cm. The current for the electrolysis is supplied from a 4 volts lead storage battery.

The polarograph of the Radiometer is furnished with a special arrangement for adjusting the strength of the diffusion current when capillaries of different constants are in use, which facilitates an easy change over from one capillary to another. By this means time wasting recalculations of the curve heights obtained can be avoided. In addition the polarograph is furnished with an arrangement for regulating the height of "the jags", arising from the dropping, the so-called "damping arrangement."

The electrolysis system has been arranged by the writer as illustrated in fig. 6.

The drop electrode has been chosen of the tapering type being the most serviceable, as it is possible to analyze small quantities of fluids with it (fig. 7).

The drop capillary is fixed by means of a spring-holder, and a piece of plastic tubing attaches it to a communicating piece of glass tubing, into which a platinum thread has been inserted so as to contact

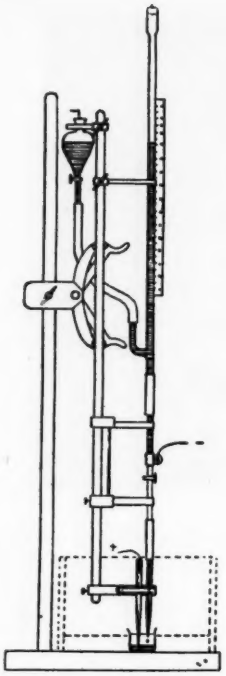

Fig. 6. Stand for polarographic analysis.

Fig. 7. Two types of drop capillaries.

the mercury in the tube. By means of another piece of plastic tubing going from the upper end of the communicating glass tube, this end is connected with a glass tube about 50 cm long with a millimeter scale and furnished with a side tube at the lower end (fig. 6). Going from this side tube there is a piece of plastic tubing, which at the other end is attached to a glass mercury reservoir that can be raised and lowered at will. The whole arrangement is fixed on to a stand with a burette-holder, so that the capillary can be easily raised and lowered without changing the level of the mercury. Reckoning from the tip of the capillary the height of the mercury is read on the long vertical glass tube and is indicated in millimeter.

Owing to the large diameter of the mercury reservoir the level of the mercury sinks only about 1 millimeter during continual dropping

for twenty-four hours, and according to Ilkovic's formula this gives an approximate change of about 0.15 % in the height of the curve. As the level is controlled and adjusted every day before the work begins and several times during the work, the level remains practically always constant.

As has already been mentioned in chapter 1, the heights of the curves are influenced by changes of temperature, in consequence of which all the analyses must be carried out at constant and the same temperature, if they are to be comparable. In order to obtain constant temperature during the analyses a thermostated water bath is used, which consists of a stainless steel container, isolated by means of asbestos and linoleum, into which there is mounted an adjustable perforated stand on which the electrolysis vessels can be placed. The temperature of the water bath is regulated by a contact thermometer and a mercury relay. A standard thermometer graduated in 1/100° C for controlling the temperature is placed into the bath, and for the purpose of analysis the temperature has been chosen according to the international standard of +25° C, the highest variation allowed being ±0°.1 C. In order to keep a homogeneous temperature the water is quickly circulated through a propeller.

Sera are denatured by heating them in a thermostated glycerole bath, constructed on the same principle as the water bath. By using glycerole it has been possible to obtain a temperature of 100° C exactly for the denaturation. The rubber plugs in the test-tubes are kept in place by strong spring clamps while the sera are being heated.

CHAPTER 6

Reagents

Absolute alcohol (99.5 vol. per cent)
Ammonia, 1.000 N (pro analysi, Kebo)
Ammonium chloride, 1.000 N (pro analysi, Kebo)
Cobalt solution: 2.6754 g. cobaltihexammine chloride + redistilled water ad 1000 ml.

Mercury for polarographic analysis: Mercury (pro analysi) is still further purified in the following manner. The mercury is poured into a fairly large bottle and then bubbled through with air for 24 hours. The mercury is then washed for 24 hours by bubbling it through with running water. The mercury, which is now washed, is purified 3 times in a Palmer apparatus and is then finally distilled three times under vacuum.

Sulphosalicylic acid solution: 50.0 gm. sulphosalicylic acid dried by desiccation (May & Baker, pro analysi) + 150 ml. redistilled water.

Buffer solution: 200 ml. ammonium chloride, 1.000 N
200 ml. cobalt solution
400 ml. ammonia, 1.000 N
1000 ml. redistilled water.

CHAPTER 7

Standardizations of the Polarographic Analysis Technique as well as the Technique for the Measurement of the Heights of the Polarographic Curves

It has already been pointed out in chapter 5, that when polarographic analyses are being carried out it is of the greatest importance to adopt accurately standardized test conditions, if absolutely reproducible values are to be obtained.

In view of the many factors influencing the height of the curves in the polarographic serum reaction, the writer has considered it necessary to work out serviceable standardizations and to adopt a simple system of control allowing of a running control of the reproducibility of the method while analytical work is in progress and also ensuring exactly comparable values from time to time.

The standardizations concerning the preparation of serum right to its polarographic analysis will be dealt with in chapter 8. In this chapter the measures will be described that are in immediate connection with the actual polarographic analysis.

These measures deal primarily with the factors associated with the polarograph itself. Here experience has shown that it is especially the linearity of recording that requires frequent control. Such deviations can be referred mainly to changes or faults in the electronic tubes of the amplifier as well as to contact defects and oxidation processes in the potentiometer. Such deviations often occur and are difficult to discover during the analysis. Daily control as described below both before and after the work of analysis has proved sufficient to eliminate this source of error.

When controlling the linearity of registration, a resistance of 0.1 megohm is connected between the anode and the cathode plugs of the

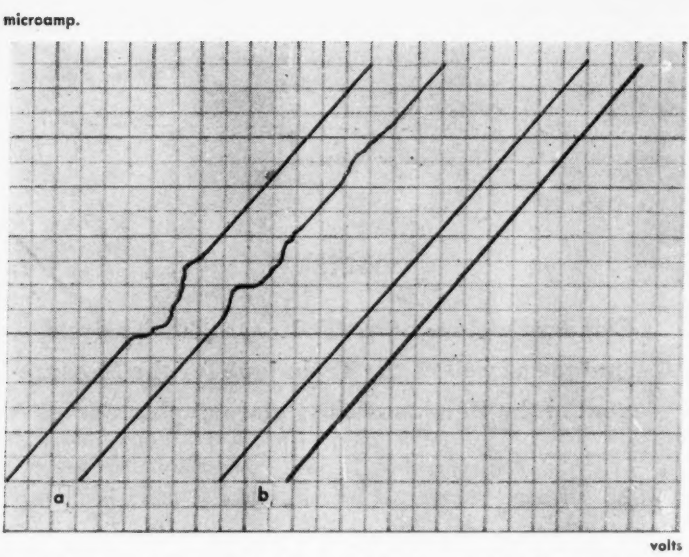

Fig. 8. Curves taken to record the control of the linearity of the registration.

a) 0—2 volts. Deviations due to imperfect contact in the potentiometer.
b) 0—2 volts. Normal curve obtained after cleaning the contact surfaces.

polarograph. When the polarograph has been switched on for about 20 minutes to get warm, a current-voltage-curve is recorded within the region of 0—2 volts. This curve must be linear.

The first few polarograms, however, occasionally show smaller or greater deviations from the linear course. The type of changes that look like small undulatory movements in the curve, usually originate from oxidation processes in the potentiometer. By turning it backwards and forwards repeatedly and thus cleaning the contact surfaces, these deviations can usually be eliminated (fig. 8). Repeated polarograms are taken until a satisfactory curve is obtained. Other types of changes may occur and must be referred to loose contacts, current leakage, faults in the electronic tubes etc.

In this work practically all the curves have been recorded by using 1 : 30 of the highest sensitivity of the galvanometer. Only in extreme cases, when the heights of the curves have been exceedingly high has 1 : 50 of the sensitivity been adopted, after which the values have been converted to the previous sensitivity.

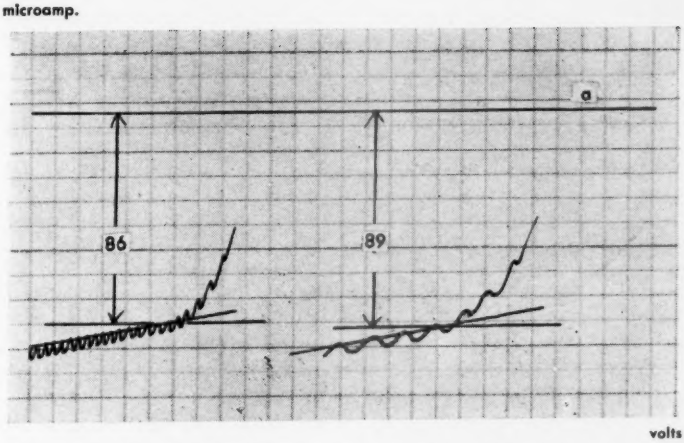

Fig. 9. Importance of the drop time when measuring the height of the curve.
a = horizontal line drawn from the apex of the double wave.

The drop time of the capillary has been controlled once a week by counting the number of drops during three ten minute periods, from which the average drop time has subsequently been reckoned. The drop control is carried out in distilled water at 0 volt.

During the three ten minute periods the drops are collected in a container and the mercury is weighed when dry. The quantity of mercury that has run out per minute is calculated. In spite of making use of the steps and measures described below, which make these controls of the dropping practically unnecessary, it has been considered wise to go on with them, since by retaining a constant drop time, it has been possible to keep the error of measure constant when measuring the curves. An explanation of this will be found in fig. 9. If the distance increases between two "jags", i. e. the drop time increases, the prospects of obtaining the exact point for the beginning of the reduction must naturally diminish. Moreover, a change in the characteristics of the dropping from the capillary indicates that it needs cleaning or should be changed.

The cleaning of the drop capillary is extremely important. It must be carried out in such a manner that after the capillary is detached, it is to be connected to an effective suction pump and emptied of its mercury contents. It is then washed first with bichromate of potassium in sulphuric acid and afterwards with distil-

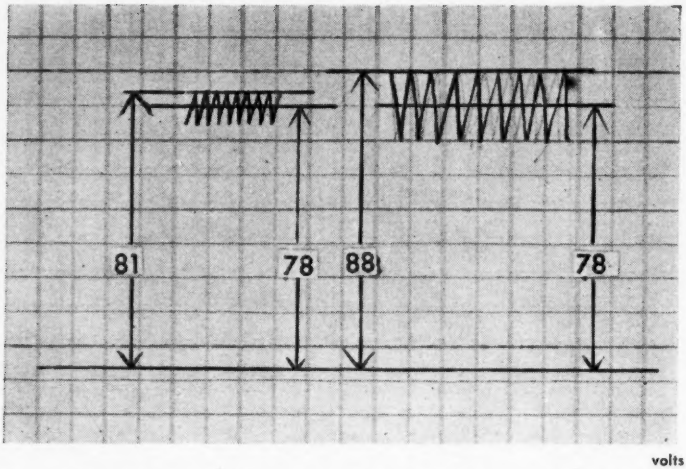

Fig. 10. Illustration of the effect of the degree of damping on the height of the polarogram when measuring the top points of the "jags".

led water, alcohol and ether. If the capillary does not then regain its normal dropping characteristics, it must be changed for a new one.

In this work the drop time has been fixed at 20 drops per minute.

In order to obtain absolutely reproducible values it has been necessary to adopt still further measures for control.

Every day both before and after the tests two samples of a standard solution consisting of 5 ml buffer solution + 0.5 ml 25 % sulphosalicylic acid solution is polarographically analysed. This mixture gives a reproducible curve without any maximum and can easily be measured, thus making it possible to see that the reproducibility does not change. If the height of the curve shows deviations from the normal value (in this work 78 h = 39 mm, see page 35), the height of the curve is corrected by readjusting the apparatus, so that the height of the curve is exactly this value. The correction is quite easy, since it can be easily determined how far the adjustment knob is to be turned to correspond to a 1 millimeter change in the height of the curve. If the deviations are greater than 2 (=1 mm), then a complete control is effected, both of the apparatus and the solutions used.

The care of the drop capillary is of the very greatest importance for the reproducibility. After testing various methods recommended

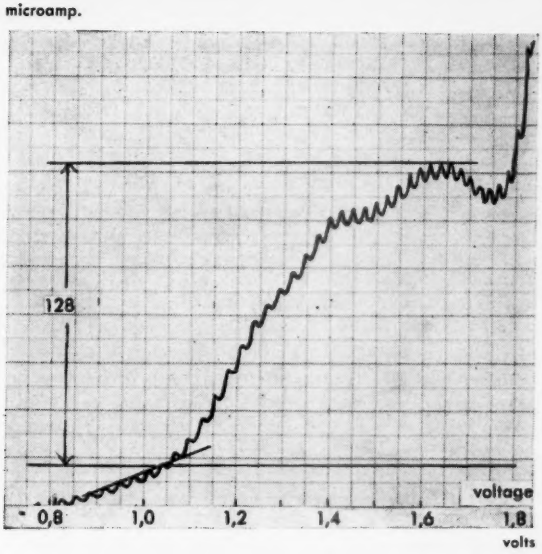

Fig. 11. Typical polarographic wave obtained from analysis of sulphosalicylic acid filtrate.

in literature, the writer has found the following to be the most reliable. The tip is dipped into concentrated nitric acid for about 30 seconds while dropping is still going on. The tip is then carefully washed in distilled water and carefully dried with a clean filter paper (N. B. not the orifice). When the capillary is not being used, dropping is prevented by stopping the flow of mercury by attaching a clamp to the lowest piece of rubber tubing. The capillary is protected by being inserted into a cork with a hole in it, fastened at the neck to a little erlenmeyer flash or something similar. This "dry" keeping of the capillary has proved a better means of preserving its properties than the suggestions made in literature of keeping it in distilled water.

By introducing these measures for control and standardization, satisfactory reproducibility and reliability are attained from a polarographic point of view, making routine analysis possible for an unlimited time.

Previous to describing the technique for measuring the serum polarograms obtained, yet another standardization must be men-

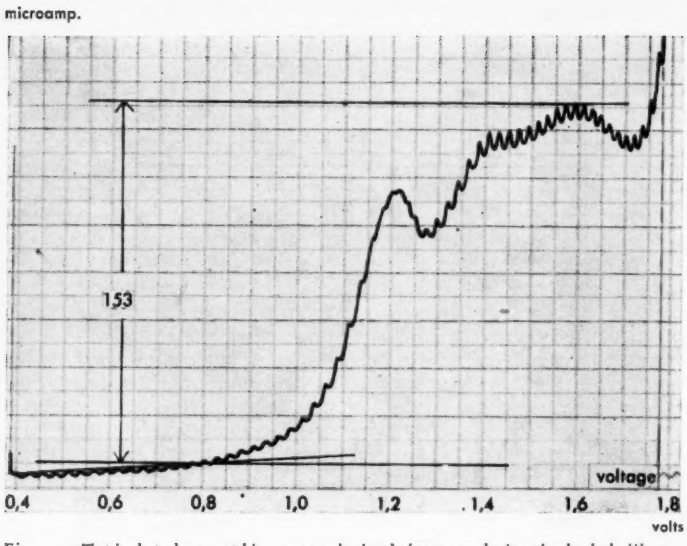

Fig. 12. Typical polarographic wave obtained from analysis of alcohol filtrate.

tioned. Since the heights of the curves are measured by taking the distance from the last regularly rising "jag" at the beginning of the reduction process to the highest point of the wave, it is necessary to regulate the oscillations resulting from the rhythmical dropping, i.e. a standardization of the heights of the "jags".

If the calculation had been made by measuring the average intensity of the current (fig. 10), this standardization would have been unnecessary and also if the heights of the "jags" had been the same in both the regions measured, which is not the case. It is more difficult to measure the average intensity of the current and in the serum polarograms this measurement involves certain practical difficulties. The writer has therefore chosen to measure according to the former principle by fixing the amplitude of the oscillations, for by so doing the variation in the height of the curve caused by the variations in the oscillations can be eliminated from time to time (fig. 10). The "jags" are always less at the beginning of the curve and they have been kept so small in these analyses that the variations can be ignored. At the top of the curve, on the other hand, they may be considerably greater, but in spite of this fact correction is seldom necessary; when this must be done, however,

it is quite easy as the polarograph of the Radiometer is equipped with a special "damping arrangement", which allows of an easy change of the heights of the "jags". The amplitude of the oscillations at the top of the curve has been fixed at 4 mm in the investigations referred to in this work.

Measurement of polarograms recorded from a sulphosalicylic acid filtrate:

The curves are recorded within the voltage range of —0.8 to —1.8.

The curve, illustrated in fig. 11, consists of a straight, gradually rising part, the residual current, and a steeply rising part caused by the reduction of the cobalt ions, which continues in the catalytic double wave without any sharp transition. The demarcation of the cobalt step being more or less vague, all the measurements have been carried out including this step, which is always of the same height. The measurement has therefore been made by drawing a straight line through the tops of the "jags" in the residual current curve recorded. A line parallel with the abscissa is drawn from the last regularly rising "jag". The top of the curve is obtained by drawing a line parallel with the base-line through the top of the highest "jag". *The distance from the lower to the upper horizontal line forms the height of the curve.* For practical reasons the heights of the curves have been expressed in units (h) of half millimeters.

Measurement of polarograms recorded from an alcohol filtrate:

The curves are recorded within the voltage range of —0.4 to —1.8.

The appearance of a curve will be seen in fig. 12. It has not been really possible to make any demarcation here of the cobalt step. In its place a maximum has been formed, after which the curve passes over into the catalytic double wave. The measurement is made as in the case of the sulphosalicylic acid filtrate curves.

Concerning the reproducibility of the method, see chapter 9.

Note: The following abbreviations will be used from now on for practical reasons.

Af = Alcohol filtrate reaction.
Sf = Sulphosalicylic acid filtrate reaction.
D = The difference Af—Sf.

CHAPTER 8

Method for Carrying out the Serum Reaction

a. Taking Blood Sample.

A blood sample for polarographic serum analysis must be taken on an empty stomach by means of a venous puncture with a dry needle. The blood must be put into a centrifugal tube 10 ml in volume. Immediately after the sample has been taken the tube must be closed with a rubber stopper and kept in room temperature for about 3 hours, and then put into a refrigerator (+4° C) till the following day, when the serum must be decanted or sucked up into a clean and dry centrifuge tube and carefully centrifuged till it is free from red blood cells. Hemolytic serum cannot be used for analysis.

b. Measurement and Dilution of Sera.

For each serum sample 4 test tubes (10×100 mm) with tightly fitting rubber stoppers are placed in a test tube stand, with 5 rows of holes with space for 10 tubes in each row. All the tubes are furnished with etched or engraved numbers, corresponding to the number on the holes in the test tube stand. The first two tubes with the same number are intended for the precipitation with sulphosalicylic acid and the other two for the precipitation with alcohol. When the method was worked out double tests were carried out on both the sulphosalicylic acid and the alcohol filtrates.

Exactly 1 ml redistilled water must be measured into each of the test tubes with calibrated pipettes, after which 0.5 ml serum is added to each tube, which is immediately closed. The pipetting must be carried out mainly according to the instructions indicated by Fuchs (82). After the admixture of the serum, the water and the serum

must be carefully mixed by gently moving the tube to and fro, and care must be taken to avoid any formation of foam. The tubes must then be placed in a test tube stand and during the heat denaturation the rubber stoppers are kept in place with strong clamps.

c. Denaturation and Precipitation of the Serum Protein.

Denaturation is carried out by means of heating, the diluted serum samples must be placed in the above mentioned stand and put down quickly into the glycerole thermostat, which has been heated up to the temperature desired. The time for the denaturation must be controlled by a stop clock. As soon as the denaturation is completed, the tubes must be immediately put down into a vessel containing water at room temperature. When they are cooled, they must be taken up, dried and replaced in their respective places in the stand. In the rows with holes which are behind the respective numbers fresh test tubes must be placed (15×70 mm) with the corresponding numbers and glass funnels (45 mm in diameter) with doubly folded round filtering papers (Munktell No OOH, 7 cm in diameter) must be inserted.

By means of a calibrated pipette an exact quantity of sulphosalicylic acid solution must be measured into each of the first two tubes with the denaturated serum. (Unless otherwise stated 1.5 ml). The rubber stoppers must be inserted, and care must be taken to see that there are no "pockets" between the wall of the tube and the stopper. The sample must then be shaken and poured into a filter. While the filtration is proceeding glass plates must be placed over the funnels. On completion of the filtering a tightly fitting rubber stopper must be immediately inserted in the tube with the filtrate.

The intended quantity of absolute alcohol must be measured to the two other tubes in each series of numbers by means of a microburette graduated in 1/100 ml and with ground-in glass taps, after which the procedure will then be as above described. (Unless otherwise stated this quantity equals 2.45 ml alcohol which corresponds to 62 % alcohol in the mixture).

In a third turn another 2 tubes for each serum filtrate must be placed in the stand and fitted up with rubber stoppers. Exactly 5.0 ml buffer solution must be measured for each tube by means of a burette, after which must be added by means of a calibrated pipette 0.5 ml sulphosalicylic acid and alcohol filtrate respectively and carefully mixed.

d. Polarographic Analysis.

For the purpose of the polarographic analysis small electrolysis vessels must be used in the shape of miniature cups, each with an exterior diameter of 18 mm and a height of 25 mm. A bottom layer of purified mercury, about 3 mm in height, must be poured into these vessels, and after this a portion of the sample that is to be analysed. The vessel must then be put down into the thermostat bath of the polarograph and the point of the drop capillary put down into the sample so that it reaches 2—3 mm below the surface of the fluid and an anode needle is put down into the layer of mercury.

When the sample has stood for the time appointed, the polarogram will be recorded within the voltage range from —0.8 to —1.8 volt when analysing the sulphosalicylic acid filtrate and —0.4 to —1.8 volt in the case of the alcohol filtrate. Should irregularities occur, the registration must be done again. The polarograms must be dated, numbers and names and polarographic data attached and pasted on to special cards, after which the curves obtained must be measured. The following day the measurements are controlled and the curves put into files in consecutive numbers.

The curves showing technical defects must be thrown away and the sample analysed afresh from the beginning. All the curves that are technically perfect have naturally been included, irrespective of any variations in the height of the curve, as it concerns the methodological work.

e. Cleaning of the Pipettes and Other Glassware.

Pipettes: When measuring serum, water and sulphosalicylic acid only calibrated pipettes that are solely used for this purpose can be employed, and after each analysis they must be rinsed and washed in running water. They must afterwards be sucked through with concentrated nitric acid, distilled water, 96 % alcohol and ether in the order given. Drying takes place by suction of air.

Other glassware: They must all be rinsed in running water after use, then cleaned in a strong NaOH solution, and afterwards in concentrated nitric acid. Finally they must be rinsed in distilled water, 96 % alcohol and then be kept in a non-dusty place till dry.

CHAPTER 9

Errors in Method and Errors in Apparatus

The error in method was determined according to the difference method. Each blood sample was divided up into two serum portions, which were then treated according to the method described in the foregoing chapter. The difference between the height of the curves for the respective serum portions was calculated. The error in method can afterwards be calculated from the mean value and the standard deviation of these differences.

In this material the error in method was determined partly for 138 blood tests, treated according to the Sf method, and partly for the same number of blood tests, treated according to the Af method (table 1).

From table 1 it will be seen that the differences of the mean values obtained ($\overline{d}$) are not significantly different from 0. This justifies the calculation of the error in method according to the above.

The error in method for the Af method is 0.65 h, and for the Sf method 0.74 h.

In consequence of the 3σ limits in table 1, and considering that in practice only integers are obtained of h, the method may give an error of one or two h in any one particular case and in extreme cases even up to 3 h.

The error in apparatus was also examined by means of the difference method. Two polarograms were recorded of the same serum portion immediately after each other. The difference was calculated as the difference between the heights of the first and the second curves.

The error in apparatus was determined on 184 blood tests, all of

which were treated according to the Sf method. The same number of blood tests were treated according to the Af method (table 2).

In the case of the Sf method the mean value of the differences differs almost significantly from zero ($P=0.016$).

In the case of the Af method no significant difference has been established ($P=0,058$).

It is obvious that there might be some factor that produces a systematic change as regards the heights of the curves as time passes, thus usually making the values of a second reading lower than those of the previous one. It is, however, worthy of note that these two methods react so differently as regards this factor.

The conditions discovered, however, can be explained by the fact that the heights of the curves for a serum filtrate drop fairly rapidly as time goes on. This decrease takes place more rapidly in the case of the sulphosalicylic filtrate than in the alcohol (as far as normal sera are concerned). This makes the decrease appear all the more markedly as regards the sulphosalicylic filtrate than it does in the case of the alcohol during the short space of time elapsing between the two analyses.

A more detailed investigation and discussion of the above mentioned factors is to be found in chapters 10—12.

The bias found will here be added to the true error of the apparatus and consequently to the error of method. This bias is, however, relatively small.

What has now been said will show the necessity of accurately fixing the point of time for the polarographic analysis.

To answer the question as to whether the polarographic curve-heights are correlated to the total protein-content of the serum, also a refractometric examination has been performed parallel with the polarographic determinations in a number of normal and pathological sera. The result will be found in Appendix I, p. 113.

TABLE 1

Calculation of the Error in Method (138 cases)

Method	$\bar{d} \pm \varepsilon_{(\bar{d})}$	σ_d	Error in method	$\pm 3 \times$ Error in method
Sf	0.015 ± 0.084	1.05	0.74	± 2.22
Af	0.080 ± 0.078	0.92	0.65	± 1.95

$\bar{d}$ = Mean value of differences.

σ_d = Standard deviation of differences.

$\varepsilon_{(\bar{d})}$ = Error of mean value.

Error in method = $\frac{\sigma d}{\sqrt{2}}$

TABLE 2

Calculation of the Error in Apparatus (184 cases)

Method	$\bar{d} \pm \varepsilon_{(\bar{d})}$	σ_d	t	P
Sf	0.152 ± 0.063	0.85	2.4	0.016
Af	0.103 ± 0.055	0.74	1.9	0.058

$t = \frac{\bar{d}}{\varepsilon_{(\bar{d})}}$

P = The probability that a t-value greater than the above may arise by chance.

PART III

Methodological Studies on Normal and Pathological Sera

CHAPTER 10

Methodological Studies on Normal Sera

a) *Testing Conditions of Deproteination. Optimal Time for the Filtration of the Samples and for the Keeping Qualities of the Filtrate both before and after the Admixture of Buffer Solution. Determination of Optimal Time for the Polarographic Analysis.*

The preliminary investigations of the reaction showed that substantial deviations arose if there were variations in the time from the moment when the precipitant was added to the denatured serum up to when the filtration took place. Considerable differences arose in the height of the curve. In order to study this state of things the following investigation was carried out. The precipitant and the diluted and denatured serum sample were carefully mixed by shaking them together for 30 seconds. Filtration was then carried out, at first immediately, then after increasing intervals of time. A time of 4 minutes was fixed for the filtration, this being sufficient to obtain a satisfactory quantity of filtrate under any circumstances. The polarographic analysis was carried out by taking two polarograms for each of two samples. The time intervals were between the filtration 0 minute after the shaking (immediately) and 20 minutes after it. The result of the investigation is to be found in table 3 (the polarographic curve heights in this and the following tables are expressed in h.).

TABLE 3

Investigation on the effect of variations in time interval between the admixture of the precipitant and the carrying out of the filtration

Filtration	Sf						Af					
	no[1] 30	no 30	no 31	no 32	no 33	no 34	no 30	no 30	no 31	no 32	no 33	no 34
immediately	97	97	94	102	96	103	121	121	119	134	119	132
after 1 min.	98	97	94	102	95	102	122	122	118	134	118	131
after 2 mins.	97	96	94	102	95	103	122	122	118	134	118	131
after 3 mins.	96	96	92	102	93	104	123	123	117	132	117	129
after 5 mins.	96	96	92	102	94	103	122	122	116	130	116	129
after 10 mins.	94	94	88	102	90	101	122	122	115	130	115	128
after 20 mins.	92	93	84	98	85	99	119	118	114	124	113	122

[1] See casuistry for numbers in this and the following tables.

It will be seen from this table that the values decline with increasing time intervals between the admixture of the precipitant and the commencement of the filtration. Up to 2 minutes after the admixture of the precipitant it is always possible to reckon on the values remaining on the same level, irrespective of any chance variations. After this time the level may be constant for different periods, even up to about 20 minutes. During the remaining time of observation (20 minutes) a continuous decline will take place. For practical reasons the writer chose to filter immediately after adding the precipitant and shaking with the serum sample.

The next thing to find out was to what extent the time taken for shaking the precipitant with the serum sample affected the heights of the polarographic curves. With this object in view, the writer investigated the values for the various times for shaking, after preparing the serum in the customary manner and after the admixture of the precipitant. Firstly the precipitant was added and the tube only turned upside down once, after which filtration was carried out, secondly the sample was shaken thoroughly for 10 and

TABLE 4

Investigation on the effect of the duration of mixing of the precipitant with the serum samples

	Sf					Af				
	no 27	no 28	no 46	no 48	no 50	no 27	no 28	no 46	no 48	no 50
without shaking	85	94	89	90	87	119	122	121	118	115
shaking for 10 secs.	86	96	90	92	88	119	122	121	118	116
shaking for 30 secs.	87	96	91	92	88	119	122	122	118	116
shaking for 1 min.	87	96	91	92	88	119	120	121	116	116
shaking for 2 mins.	87	96	91	91	88	119	122	121	118	116

30 seconds and for 1 and 2 minutes. The result of the investigation will be found in table 4.

It will be seen from the table—apart from the case in which the tube was only turned upside down—that the values are the same for all the times for shaking between 10 seconds and 2 minutes. In order to make sure that the mixing is satisfactory in every case, the writer always gives a thorough shaking for 30 seconds.

It is also of importance to know the keeping qualities of the filtrate as well as of the mixture of the filtrate and the buffer solution.

Investigations in order to ascertain this were carried out according to the following description. When the samples had been prepared in the customary manner, 0.5 ml. filtrate was measured off and mixed with 5 ml. buffer solution. Duplicates were taken of both the sulphosalicylic acid and the alcohol filtrates. Controls were taken at fixed time intervals of 5 minutes parallel with tests for each time interval. The samples were kept in room temperature (+ 20° C) and in closed vessels, thus preventing the effect of any evaporation. One polarogram was recorded of every filtrate. The figures given in table 5 are the mean values obtained with 2 different filtrate samples.

As will be seen from the table, there was no change in the heights of the polarographic curves up to 4 hours, when a mixture of

TABLE 5

Investigation on the keeping qualities of the filtrate after the admixture of buffer solution (stored in a closed vessel)

Sample no	Time[1]	S. Ö. Man age 33		A. F. Woman age 19		K. Ö. Woman age 57	
		Sf	Af	Sf	Af	Sf	Af
1	5	110	131	95	122	98	124
	10	110	131	95	122	98	124
2	5	110	131	95	122	98	123
	20	110	131	95	122	97	124
3	5	110	131	95	122	98	124
	30	110	131	95	122	98	124
4	5	110	131	95	122	98	124
	60	110	131	95	122	97	122
5	5	110	131	95	122	98	124
	120	112	133	95	122	98	124
6	5	110	131	95	122	98	123
	240	112	133	97	124	99	123

[1] Measured in minutes from the time when the filtrate was mixed with the buffer solution up to the commencement of the polarographic analysis.

filtrate and cobalt solution was kept in room temperature in a closed vessel. After 4 hours there seemed to be a tendency to a slight increase.

The keeping qualities of the original filtrate were examined in an analogous manner. The filtrates obtained were kept for different periods of time from when the filtration was complete up to when the buffer solution was added. The intervals of time were increased from 5 minutes to 24 hours. Each analysis was controlled by a similar one, which was carried out at a fixed interval of ten minutes. The result of this test series is collected in table 6.

TABLE 6

Investigation on the keeping qualities of the filtrates

Sample no	Time[1]	Sf				Af			
		no 32	no 33	no 34	no 35	no 32	no 33	no 34	no 35
1	5 m	98	90	96	90	132	121	133	121
	10 m	98	91	96	90	130	120	132	120
2	10 m	98	90	96	90	130	120	132	121
	15 m	98	90	96	90	130	120	132	121
3	10 m	98	90	96	90	130	121	132	122
	20 m	98	90	97	90	130	120	132	120
4	10 m	98	91	96	90	130	120	131	120
	30 m	98	90	95	90	130	120	132	120
5	10 m	99	90	96	90	130	120	132	120
	1 h	98	90	96	89	129	119	131	120
6	10 m	98	90	96	90	130	120	132	121
	2 h	98	90	96	90	130	120	132	120
7	10 m	98	90	96	90	130	120	132	120
	3 h	98	90	96	91	129	120	131	120
8	10 m	98	90	96	90	130	120	132	120
	24 h	98	90	96	90	130	119	132	120
9	10 m	98	90	96	91	130	119	132	120
	24 h	98	90	96	90	130	120	132	120

[1] Measured in minutes and hours respectively from time of filtration till when the buffer solution was added, after which immediate polarographic analysis was performed. Samples nos 1—8 kept at room temperature. Sample no 9 kept in a refrigerator at +4° C.

TABLE 7

Determination of the optimal time for the polarographic analysis after transferring the sample to the electrolysis vessel

Time[1]	Sf					Af				
	S.Ö.	T.P.	A.F.	I.B.	G.S.	S.Ö.	T.P.	A.F.	I.B.	G.S.
2	94	100	98	107	106	118	118	118	117	118
5	94	100	98	106	106	118	117	118	116	117
10	94	100	98	106	106	117	117	118	116	117
15	94	100	98	106	106	117	117	117	116	117
20	92	98	98	105	106	118	116	116	115	115
25	92	97	96	102	105	117	114	116	115	115
30	—	98	95	103	105	116	114	115	114	115
35	91	98	92	102	103	116	113	114	113	114
40	89	97	92	102	103	116	114	114	113	113
45	89	96	93	102	99	115	113	114	113	112
50	89	95	91	98	99	114	113	113	112	112
55	88	95	91	98	99	114	112	113	112	112
60	86	95	91	98	98	114	111	112	111	112

[1] Measured in minutes from the transference of the test to the electrolysis vessel up to the commencement of the polarographic analysis.

Samples nos. 1—8 were kept at room temperature, whereas sample no 9 was put into a refrigerator (+ 4° C). All the analyses were carried out with duplicates of sulphosalicylic acid and alcohol filtrates respectively, but with one polarogram for each filtrate. The figures given in the table are the mean values of the heights of curves of two filtrates.

As will be seen from table 6, the polarographic values vary within

normal limits. Hence the investigation shows that the filtrates from normal sera can be kept for 24 hours in a refrigerator or at room temperature.

The following investigation was carried out in order to clear up the question as to the most suitable point of time for the polarographic analysis after the filtrates, when mixed with the buffer solution, had been transferred to the electrolysis vessels.

Serum from five healthy test subjects was diluted, denatured by heat for 12.5 mins. at 80° C in a glycerole thermostat and was precipitated with sulphosalicylic acid and alcohol respectively (1.5 ml. 25 per cent sulphosalicylic acid solution and 2.45 ml. alcohol respectively) as has already been described in chapter 8. A quantity of 0.5 ml. of the filtrate was mixed with 5 ml. buffer solution, after which a polarogram was taken of every sample at regular intervals: 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes after the sample had been poured into the electrolysis vessel. The results of the polarographic analyses are to be found in table 7.

In all the cases two precipitation tests were carried out with sulphosalicylic acid and alcohol respectively from every test subject.

It will be seen from the table that both the Sf. and the Af. values remain constant up to 15 minutes after the transference of the sample to the electrolysis vessel, after which the values slowly decline. In some few cases the level remains constant a little longer. For practical reasons the writer fixed the time to 5 minutes, thus making it possible to eliminate the risk for too low values.

b) *Investigation on the Effect of the Temperature and the Time of Denaturation in Normal Sera.*

Earlier writers who chose to carry out the denaturation of sera by means of heat made use of boiling water baths. No systematic report on the conditions prevailing when denaturation was carried out at different temperatures has been found in literature. There is really no pertinent reason for choosing 100° C as the most suitable temperature for denaturation, and therefore the writer has considered it fit to study the reaction within the range of + 40° C— + 100° C.

The tests were carried out according to the method described in chapter 8, care being taken to follow the optimal conditions for the carrying out the reactions which are prescribed in the previous chapter 10 a). All the analyses were carried out with duplicates of

both the sulphosalicylic acid and alcohol filtrates with one polarogram from each sample. Hence the figures given in table 8 are the mean values of 2 polarograms.

The denaturation was carried out in a glycerole thermostat, allowing of an accuracy of ±0.05 ° C. The intervals were chosen at 10° C, in consequence of which the samples of normal serum were denatured at 40, 50, 60, 70, 80, 90 and 100° C. The investigation was carried out with different denaturation times, viz. 5, 10, 15 and 20 minutes. The results of the denaturation tests are to be found in tables 8 and 9 as well as in figs. 13—16. Each three analyses with sulphosalicylic acid and alcohol filtrate were carried out with durations of 5 and 20 minutes respectively. Each five analyses of the respective filtrates were also carried out with durations of 10 and 15 minutes.

It will be seen from the diagrams that practically speaking constant polarographic values were obtained within the temperature range of 40°—50° C with all durations of the denaturation. Between 50° and 70° C there was a considerable decrease in the heights of the curves, as was also the case with all durations of the denaturation. At a denaturation time of 5 minutes, the heights of the curves were equally high from 80° to 100° C. Between 70° and 80° C there was only a very slight decline. In the 10 and 15 minute intervals it was practically the same thing. In both cases the polarographic values were constant från 70° up to 90° C. Between 90° and 100° C there was another rise in the heights of the curves. Here it will be seen that the increase in the height of the curve was more pronounced in the 15 than in the 10 minute interval. In the 20 minute denaturation the polarographic values remained constant between 70° and 80° C, after which they continued to rise up to 100° C. The rise was practically of the same magnitude as in the former time interval.

If D is indicated graphically, the curves obtained will appear as shown in fig. 17. For all time intervals D is about constant within the temperature range of 40°—50° C. Between 50° and 60° C there is a considerable increase in D, because the decline in the values of the sulphosalicylic acid is comparatively greater than in those of the alcohol. Between the temperatures of 60° and 70° C there is a small decline in D in the 10, 15 and 20 minute intervals, but a slight rise in the 5 minute interval. Between the temperatures of 70° and 100° C the values of D appear unchanged practically speaking within all the temperature intervals with the exception of the 20 minute one, where D increases somewhat between 90° and 100° C. Hence, taken as a whole, D remains constant within the

last temperature range, while the values for the sulphosalicylic acid and the alcohol present a rise, which runs almost parallel, whereas the decline between 50° and 60° C is more pronounced in the case of the sulphosalicylic acid than in that of the alcohol filtrate reaction.

Fig. 18 presents a graph of the time and temperature ranges, within which constant sulphosalicylic acid and alcohol filtrate values were obtained when denaturation took place within 40°—100° C, and at intervals of 5, 10, 15 and 20 minutes. As will be seen from the figure, when there is an increase in the time for denaturation, this constant range will move towards a lower temperature.

c) *Investigation on the Keeping Qualities of Normal Sera in Storage.*

In order to investigate the keeping qualities of a number of normal sera, firstly when kept at room temperature (+20° — +27° C) and secondly in a refrigerator (+4° C), an analysis of four sera was carried out, firstly on the day after the blood sample was taken and secondly 2, 4, 8 and 14 days after.

As will be seen from table 10, when sera are kept at room temperature for only two days after taking the sample there is a fall in the polarographic values, which continues until 8 days after the test. Even D decreases continuously.

A fortnight after the blood sample was taken, sera "A.H.", "S.Ö." and "S.G." that were stored at room temperature showed signs of putrefaction, which was most pronounced in serum "S.Ö". The rise in the polarographic values which took place during the putrefaction is also to be found in the "B.S". serum in the sulphosalicylic acid reaction. This serum showed no manifest signs of putrefaction. In the "S.Ö." serum, which was most putrefied, the rise in the polarographic value was most strongly pronounced. In the alcohol filtrate the rise in the polarographic curves only appeared in the "S.Ö". serum, while in all the others there were further falls. The considerable fall in D is due to the rise in the sulphosalicylic acid reaction.

During the whole time that sera are kept in refrigerators, a slow fall in the values is going on in both the sulphosalicylic acid and alcohol filtrates, it being most pronounced in the latter. The decrease in D will therefore chiefly be due to the falling curves from the alcohol filtrates.

TABLE 8

Investigation on the effect of the duration and the temperature of denaturation on the heights of the polarograms in normal sera

Time[1]	Case	Sf							Af						
		40°	50°	60°	70°	80°	90°	100°	40°	50°	60°	70°	80°	90°	100°
5	10	140	137	122	106	102	102	102	154	153	146	127	123	123	123
	13	130	130	116	96	91	91	91	146	145	143	128	122	122	122
	14	135	135	120	92	89	89	89	154	151	146	123	116	116	115
	M	135	134	119	98	94	94	94	151	149	145	126	120	120	120
10	9	125	129	110	99	99	99	108	150	148	142	128	128	128	139
	12	139	133	108	90	90	90	92	145	144	135	114	114	114	118
	19	137	136	113	90	90	90	93	151	147	134	114	114	114	120
	21	131	130	114	95	90	90	96	154	152	145	119	119	119	119
	20	127	130	107	91	91	91	98	143	141	134	113	113	114	120
	M	132	131	110	93	92	92	97	149	146	138	118	118	118	123
15	11	135	134	108	102	102	102	108	155	148	135	126	126	126	136
	18	136	133	103	90	90	90	106	151	149	131	114	114	114	125
	22	134	133	105	91	91	91	109	151	149	134	118	118	118	128
	23	142	139	110	99	99	99	110	153	150	138	118	118	118	134
	24	154	158	119	103	103	104	120	160	161	149	124	124	124	147
	M	140	139	109	97	97	97	111	154	151	137	120	120	120	134
20	8	161	155	119	104	103	103	115	162	159	145	127	127	127	144
	15	139	137	107	101	101	107	118	141	139	131	123	123	126	139
	16	131	130	106	94	94	101	110	138	137	132	116	116	120	131
	M	144	141	111	99	99	104	114	147	145	136	122	122	124	138

[1] Duration of denaturation in minutes. M = Mean value.

TABLE 9

D values for normal sera at various durations and temperatures of denaturation

Time[1]	Case	D						
		40°	50°	60°	70°	80°	90°	100°
5	10	14	16	24	21	21	21	21
	13	16	15	27	32	31	31	31
	14	19	16	26	31	27	27	26
	M	16	16	26	28	26	26	26
10	9	25	19	32	29	29	29	31
	12	6	11	27	24	24	24	26
	19	14	11	21	24	24	24	27
	21	23	22	31	24	24	24	23
	20	16	11	27	22	22	23	22
	M	17	15	28	25	25	25	26
15	11	20	14	27	24	24	24	26
	18	15	16	28	24	24	24	19
	22	17	16	29	27	27	27	19
	23	11	11	28	20	20	20	24
	24	6	3	30	21	21	20	27
	M	14	12	28	23	23	23	23
20	8	1	4	26	23	24	24	29
	15	2	2	24	22	22	19	21
	16	7	7	26	22	22	19	21
	M	3	4	25	22	23	21	24

[1] Duration of denaturation in minutes. M = Mean value.

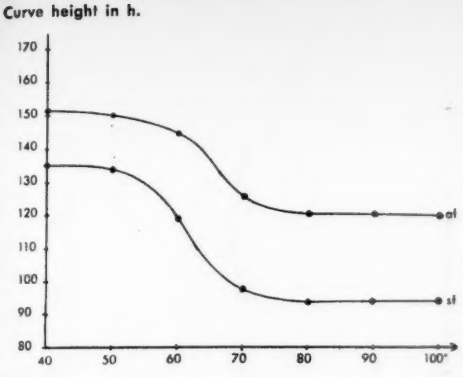

Fig. 13. Normal cases. Tests with denaturation for 5 mins. at different temperatures. Mean values.

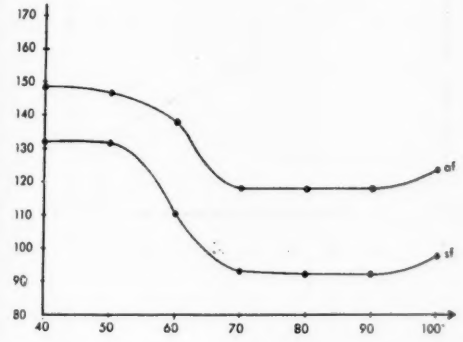

Fig. 14. Normal cases. Tests with denaturation for 10 mins. at different temperatures. Mean values.

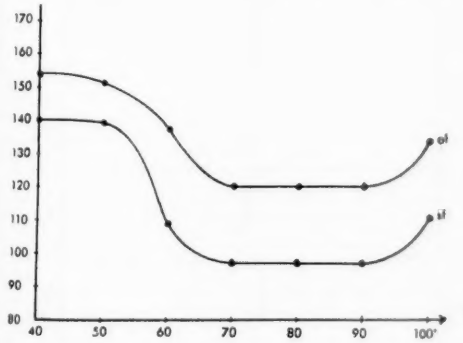

Fig. 15. Normal cases. Tests with denaturation for 15 mins. at different temperatures. Mean values.

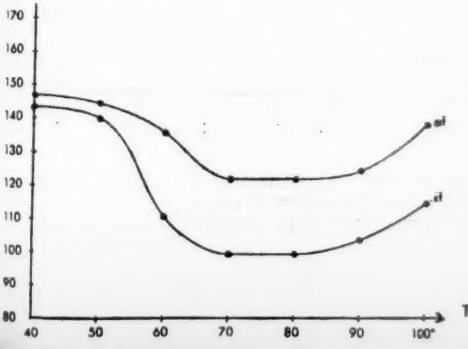

Fig. 16. Normal cases. Tests with denaturation for 20 mins. at different temperatures. Mean values.

Fig. 17. Mean values of D for normal cases. Tests with denaturation during 5—20 minutes at different temperatures.
(The curves correspond to figs. 13—16.)

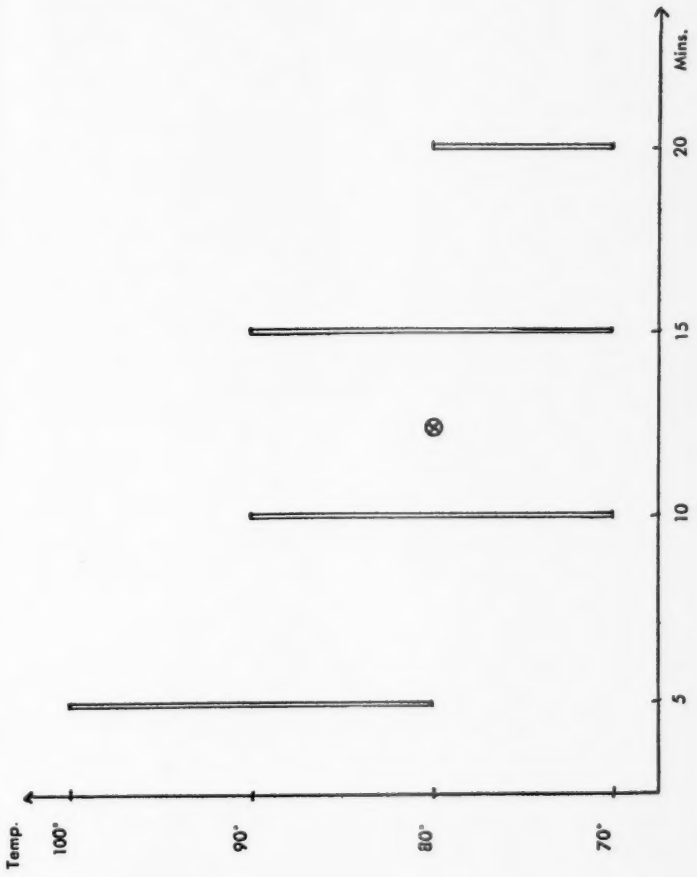

Fig. 18. Graph of the time and temperature ranges within which constant sulphosalicylic acid and alcohol filtrate values were obtained when denaturation took place during 5, 10, 15 and 20 minutes. The optimal point for stable values being at 12.5 minutes and 80° C.

TABLE 10

Investigation on the keeping qualities of normal sera when stored in a refrigerator and at room temperature

a) Stored at room temperature (+ 20° — + 27° C)									
No. of days and nights[1]	Sf				Af				D[2]
	A. H.	S. Ö.	B. S.	S. G.	A. H.	S. Ö.	B. S.	S. G.	
1	107	106	100	98	128	129	126	122	+ 23.5
2	105	104	102	98	126	129	130	120	+ 24.0
4	103	103	92	94	123	125	115	116	+ 21.8
8	102	102	91	90	120	120	113	114	+ 20.5
14	107	211	95	112	112	208	109	108	(+ 3.0)
b) Stored in a refrigerator at + 4° C									
No. of days and nights[1]	Sf				Af				D[2]
	A. H.	S. Ö.	B. S.	S. G.	A. H.	S. Ö.	B. S.	S. G.	
1	107	107	101	98	128	128	131	122	+ 24.0
2	107	106	102	98	128	127	129	122	+ 23.3
4	108	104	102	99	128	124	131	121	+ 22.8
8	108	105	102	97	129	125	125	122	+ 22.3
14	109	101	102	98	128	121	124	120	+ 20.8

[1] "Number of days and nights" = number of days and nights between the taking of the blood sample and the analysis.

[2] The D values are calculated as the difference between the mean values.

CHAPTER 11

Methodological Studies on Pathological Sera

a) *Testing Conditions of Deproteination. Optimal Time for the Filtration of the Samples and for the Keeping Qualities of the Filtrate both before and after the Admixture of Buffer Solution. Determination of Optimal Time for the Polarographic Analysis.*

In chapter 10 a) methodological studies were carried out on normal sera. The corresponding investigation of pathological sera was carried out, partly with sera from cancer patients and partly from patients with various acute infectious diseases. The object of this classification was to study whether it was possible to observe any difference between cancer sera on the one hand and sera in infectious or inflammatory diseases on the other. Even if no specific difference was really to be expected, the writer nevertheless considered it desirable to study whether the general properties of the decompo sition products are similar or not. Such a similarity of properties would to a certain extent support the opinion that the polarographically active substances are the same as well in normal sera as in cancerous, infectious and inflammatory sera.

The investigations were carried out according to the same principles and the same method as were adopted in chapter 10 a).

Tests were carried out with times varying from when the precipitant was added to when the filtration was carried out. The result of the investigation is collected in table 11.

From this it will be seen that the values decrease with increasing time intervals from the time of the admixture of the precipitant to the beginning of the filtration. The decrease seems to begin fairly quickly after the admixture of the precipitant, but the change during the first few minutes is so slight that the error in the deter-

TABLE 11

Investigation on the effect of variations in time interval between the admixture of the precipitant and the carrying out of the filtration

a) Cancer cases

Filtration	Sf						Af					
	no 86	no 87	no 88	no 89	no 90	no 91	no 86	no 87	no 88	no 89	no 90	no 91
immediately	114	207	138	190	185	192	123	190	144	200	134	134
after 1 min.	114	208	138	191	185	192	122	190	143	200	134	133
after 2 mins.	112	206	136	188	184	191	121	188	141	198	132	133
after 3 mins.	110	205	135	186	183	190	119	186	137	195	128	132
after 5 mins.	109	203	133	185	179	188	116	182	136	191	125	129
after 10 mins.	104	200	127	182	176	184	111	177	134	189	123	124
after 20 mins.	102	197	124	180	173	179	111	175	130	187	119	120

b) Infectious cases

Filtration	Sf						Af					
	no 77	no 78	no 79	no 80	no 81	no 82	no 77	no 78	no 79	no 80	no 81	no 82
immediately	160	175	198	157	140	208	164	190	207	156	161	200
after 1 min.	159	175	198	158	140	207	164	190	207	156	160	199
after 2 mins.	159	174	197	158	138	206	163	189	207	156	158	198
after 3 mins.	158	172	196	156	135	202	163	187	206	153	157	196
after 5 mins.	156	170	192	152	133	199	159	184	201	150	155	195
after 10 mins.	152	164	188	149	130	198	155	181	196	147	152	190
after 20 mins.	149	162	185	146	129	196	151	177	195	144	160	189

TABLE 12

Investigation on the effect of the duration of mixing of the precipitant with the serum samples

a) Cancer cases

	Sf					Af				
	no 87	no 86	no 88	no 89	no 90	no 87	no 86	no 88	no 89	no 90
without shaking	207	114	138	190	185	190	123	144	200	134
shaking for 10 secs.	207	114	137	190	185	188	122	143	200	133
shaking for 30 secs.	207	113	137	191	186	190	123	144	200	134
shaking for 1 min.	207	114	138	191	186	188	123	144	201	134
shaking for 2 mins.	207	115	138	191	186	188	123	143	202	133

b) Infectious cases

	Sf					Af				
	no 77	no 78	no 79	no 80	no 81	no 77	no 78	no 79	no 80	no 81
without shaking	160	175	198	157	140	164	190	207	156	161
shaking for 10 secs.	161	175	198	156	140	164	190	208	156	162
shaking for 30 secs.	161	175	197	157	140	165	190	207	156	162
shaking for 1 min.	160	175	197	157	141	164	190	208	156	162
shaking for 2 mins.	160	175	197	157	141	164	190	207	156	162

mination is of no greater magnitude than that of the method. This applies just as much to cancer as to sera from infectious diseases.

The next thing to be investigated was the extent to which the time for the shaking of the precipitant with the serum sample affected the heights of the polarographic curves. The result of the test is to be found in table 12. It proved that the values are the same for all the times for shaking between 10 seconds and 2 minutes.

TABLE 13

Investigation on the keeping qualities of the filtrate after the admixture of buffer solution (stored in a closed vessel). Sera from cancer cases

Sample no	Time[1]	Cancer case no 87		Cancer case no 90		Cancer case no 89	
		Sf	Af	Sf	Af	Sf	Af
1	5	207	193	185	134	190	200
	10	207	193	185	134	189	200
2	5	208	193	185	133	189	201
	20	207	194	185	134	189	200
3	5	207	194	183	134	190	200
	30	207	193	184	133	189	199
4	5	206	192	185	134	189	199
	60	206	193	184	133	191	200
5	5	207	193	185	134	190	200
	120	206	194	185	134	189	201
6	5	207	194	185	133	189	200
	240	207	193	185	133	190	200

[1] Measured in minutes from the time when the filtrate was mixed with the buffer solution up to the commencement of the polarographic analysis.

The keeping qualities of the filtrate after it had been mixed with buffer solution were then tested. The samples were kept in closed vessels. Table 13 shows that in the case of cancer sera there is no change in the heights of the curves, when the filtrate is kept up to 4 hours. When corresponding tests were carried out with sera from acute infectious diseases (table 14), the result was the same.

The keeping qualities of the original filtrate were investigated according to the method described in chapter 10 a). The values in table 15 show that the sulphosalicylic acid filtrate of both cancer

TABLE 14

Investigation on the keeping qualities of the filtrate after the admixture of buffer solution (stored in a closed vessel). Sera from cases with infectious diseases

Sample no	Time[1]	Infectious case no 211		Infectious case no 212		Infectious case no 213	
		Sf	Af	Sf	Af	Sf	Af
1	5	230	215	198	193	160	159
	10	230	213	198	193	160	159
2	5	230	215	197	192	160	159
	20	230	215	198	193	160	159
3	5	230	215	198	193	161	159
	30	227	213	198	193	161	159
4	5	230	213	197	193	160	159
	60	230	213	198	192	160	158
5	5	230	215	198	193	160	159
	120	230	215	198	193	160	157
6	5	230	213	197	192	161	159
	240	229	213	197	193	160	158

[1] Measured in minutes from the time when the filtrate was mixed with the buffer solution up to the commencement of the polarographic analysis.

and infectious sera do not change their polarographic activity when kept as long as 24 hours in a refrigerator or at room temperature. When the alcohol filtrate was investigated, no definite change took place when it was kept up to 3 hours at room temperature. On the other hand alcohol filtrate shows a decrease after 24 hours when kept at room temperature and in a refrigerator.

The results of the tests made in order to ascertain the most suitable time for the polarographic analysis of the filtrates mixed with buffer

TABLE 15

Investigation on the keeping qualities of the filtrates

Sample no	Time[1]	Sf						Af					
		Cancer cases			Infectious cases			Cancer cases			Infectious cases		
		no 87	no 90	no 89	no 214	no 215	no 82	no 87	no 90	no 89	no 214	no 215	no 82
1	5 m	207	185	190	163	237	208	193	134	200	158	232	200
	10 m	207	185	190	162	237	207	193	134	200	158	232	200
2	10 m	207	185	190	162	237	208	193	134	200	158	232	200
	15 m	206	185	191	162	238	208	193	135	201	158	232	199
3	10 m	206	185	190	162	237	208	193	134	201	158	233	200
	20 m	207	184	189	162	237	208	193	133	200	158	232	200
4	10 m	207	185	189	163	237	208	194	134	200	158	232	200
	30 m	206	185	189	162	237	208	193	133	199	158	232	201
5	10 m	207	185	190	163	237	208	192	134	199	158	232	201
	1 h	206	185	190	163	237	208	192	135	200	158	232	200
6	10 m	206	184	190	162	237	207	193	135	199	158	232	200
	2 h	207	184	191	162	237	208	193	134	200	157	230	200
7	10 m	207	186	190	162	237	208	193	135	200	158	232	200
	3 h	206	185	189	162	237	208	193	133	199	157	230	200
8	10 m	207	185	190	162	237	208	193	134	200	158	232	200
	24 h	206	185	188	162	237	207	191	129	198	152	227	196
9	10 m	207	184	200	163	237	208	192	134	199	158	232	200
	24 h	206	184	198	163	237	207	192	130	197	152	230	198

[1] Measured in minutes and hours respectively from time of filtration till when the buffer solution was added, after which immediate polarographic analysis was performed. Samples nos 1—8 kept at room temperature. Sample no 9 kept in i refrigerator at +4° C.

TABLE 16

Determination of the optimal time for the polarographic analysis after transferring the sample to the electrolysis vessel

Time[1]	Sf						Af					
	Cancer cases			Infectious cases			Cancer cases			Infectious cases		
	no 87	no 89	no 90	no 79	no 77	no 78	no 87	no 89	no 90	no 79	no 77	no 78
2	207	190	185	198	160	175	193	200	134	206	164	190
5	207	190	184	197	160	176	193	202	135	206	164	190
10	205	187	184	196	159	175	190	200	133	204	162	189
15	202	183	182	192	157	171	187	195	130	201	159	188
20	200	180	179	187	155	168	185	192	130	197	157	187
25	197	177	175	185	150	165	180	187	124	193	153	183
30	195	175	171	184	148	162	180	187	122	191	153	179
35	193	173	168	180	147	160	177	183	120	188	153	175
40	192	170	166	177	146	157	175	183	118	186	150	170
45	188	169	165	175	146	156	173	180	117	184	147	166
50	187	167	164	172	144	153	173	177	115	180	144	164
55	183	167	163	167	142	150	167	175	113	177	140	163
60	183	165	161	167	142	148	167	173	111	178	137	161

[1] Measured in minutes from the transference of the test to the electrolysis vessel up to the commencement of the polarographic analysis.

solution after they had been transferred to the electrolysis vessel are to be found in table 16. From this it will be seen that the values of both the sulphosalicylic acid and the alcohol filtrates remain for the most part constant up to 5 mins. after being transferred, after which they drop. The result is the same with both cancer and infectious sera.

TABLE 17

Investigation on the effect of the duration and the temperature of denaturation on the heights of the polarograms in cancer sera

Time[1]	Case	Sf							Af						
		40°	50°	60°	70°	80°	90°	100°	40°	50°	60°	70°	80°	90°	100°
5	221	208	201	191	183	182	198	218	151	150	149	167	178	190	200
	224	187	187	182	168	166	172	190	125	125	114	132	147	160	175
	225	195	190	186	180	173	194	223	176	173	167	166	165	192	210
10	230	188	186	176	177	181	189	190	153	156	156	184	184	200	191
	231	154	154	143	123	118	118	135	147	147	141	131	128	128	131
	232	170	170	161	151	147	158	165	154	154	152	150	146	157	165
	233	189	181	170	165	178	186	190	162	164	160	155	161	173	175
15	234	184	185	177	186	188	196	193	157	154	154	179	186	200	201
	236	170	176	162	143	148	190	201	152	152	153	148	143	160	169
	245	200	196	193	190	185	230	275	193	190	181	179	174	235	238
	246	185	186	177	176	177	205	220	167	164	163	174	175	199	202

[1] Duration of denaturation in minutes.

As far as the investigation of pathological sera is concerned, no definite difference was observed between cancer sera and those of acute infectious diseases. It was possible to record the polarographic curves with satisfactory accuracy up to 5 minutes after the tests had been transferred into the electrolysis vessels.

b) *Investigation on the Effect of the Temperature and the Time of Denaturation in Pathological Sera.*

Investigations have been carried out on pathological sera analogous to those described in chapter 10 b). The temperature range

TABLE 18

D values for cancer sera at varying durations and temperatures of denaturation

Time[1]	Case	D						
		40°	50°	60°	70°	80°	90°	100°
5	221	−57	−51	−42	−16	−4	−8	−18
	224	−62	−62	−68	−36	−19	−12	−15
	225	−19	−17	−19	−14	−8	−2	−13
10	230	−35	−30	−20	+7	+3	+11	+1
	231	−7	−7	−2	+8	+10	+10	−4
	232	−16	−16	−9	−1	−1	−1	±0
	233	−27	−17	−10	−10	−17	−13	−15
15	234	−27	−31	−23	−7	−2	+4	+8
	236	−18	−24	−9	+5	−5	−30	−32
	245	−7	−6	−12	−11	−11	+5	−37
	246	−18	−22	−14	−2	−2	−6	−18

[1] Duration of denaturation in minutes.

studied has been 40° and 100° C, the durations being 5, 10 and 15 minutes.

In the 5 minute duration 3 series of analyses of cancer sera were carried out with both sulphosalicylic acid and alcohol filtrate reaction. In the 10 and 15 minute durations 4 series of analyses were carried out in each.

The results obtained are to be found in tables 17 and 18 and also in figs. 19—32.

If the three cancer cases with 5 minute denaturation are examined, it will be found a drop in the Sf values to a temperature of 80° and then they rise again. The rising part of the curve is steeper than the falling. As regards the Af values, there seems to be a slight drop in the values up to 60°, after which the values rise rapidly.

With regard to the 10 minute denaturations with cancer sera, it will be found that in 3 of the 4 cases there is a fairly slow drop in the values up to a temperature of ca. 70°—80°, both in the Sf and the Af. This drop is followed at higher temperatures by slowly rising values. In the fourth case the Sf values proceed in the manner just mentioned, while the Af values, after having been constant up to ca. 60°, show a rise, which at 100° tends to go over to a drop.

In the 15 minute interval 3 of the 4 cancer cases investigated show decreasing Sf values again up to ca 70°—80°, after which there is a more or less considerable rise. In the fourth case the Sf values remain constant up to 60° and then rise.

Generally speaking the Af values show in 2 of the cases the same result as the Sf values in the three first mentioned cases. In the two others the Af values are the same as the Sf in the fourth case, which has just been mentioned.

The D values are to be found in figs. 30—32, where the dashed curves represent the average values of the separate curves in the respective figures. It will be seen that the D values remain fairly constant in the 2—3 lowest temperature intervals, after which there is a rather considerable rise, which after having reached a maximum value again drops at the highest temperatures.

From the figures it will further be seen that the temperature at the maximum mentioned seems to drop with the increasing time of denaturation. At the 5 minute denaturation it is at ca. 90° ; at 10 minutes it is probably at ca. 70°—90° and at 15 minutes ca. 70°—80°.

c) *Investigation on the Keeping Qualities of Pathological Sera in Storage.*

The keeping qualities when stored of a number of cancer sera as well as sera from patients with infectious diseases have been investigated in exactly the same manner as was normal sera in chapter 10 c). The results of the analyses are to be found in tables 19 and 20.

When stored at room temperature there is a decrease in the polarographic values after only about 24 hours, and this continues during the first 8 days and nights that the observation lasts. This state is the same to both cancer and infectious sera. When analysed 14 days after the blood sample was taken, all the sera were found to show a more or less pronounced rise in the polarographic values and at the same time a state of putrefaction was also noticed in the sera.

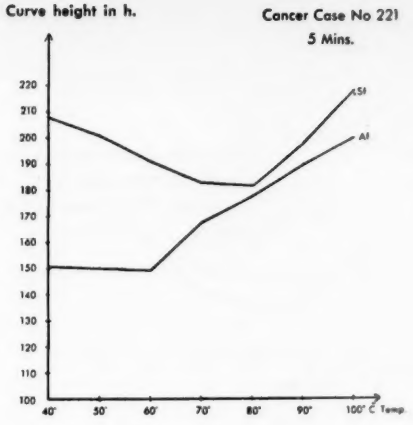

Fig. 19. Cancer case. Tests with denaturation for 5 mins. at different temperatures.

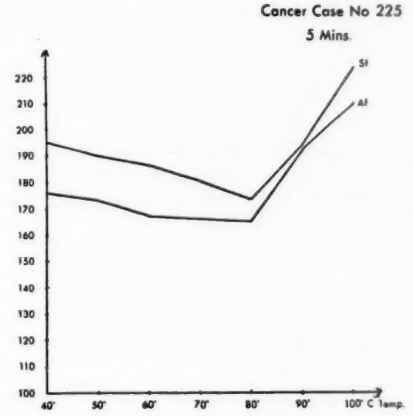

Fig. 20. Cancer case. Tests with denaturation for 5 mins. at different temperatures.

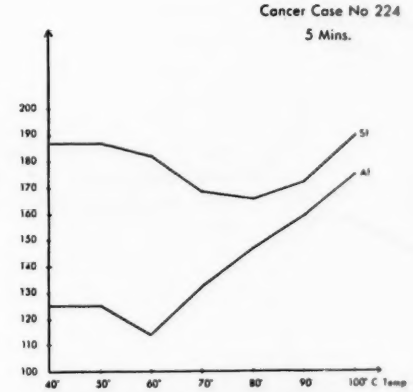

Fig. 21. Cancer case. Tests with denaturation for 5 mins. at different temperatures.

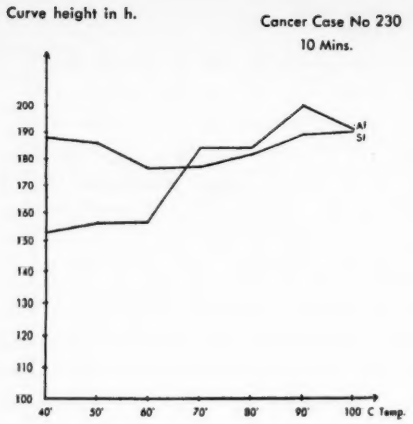

Fig. 22. Cancer case. Tests with denaturation for 10 mins. at different temperatures.

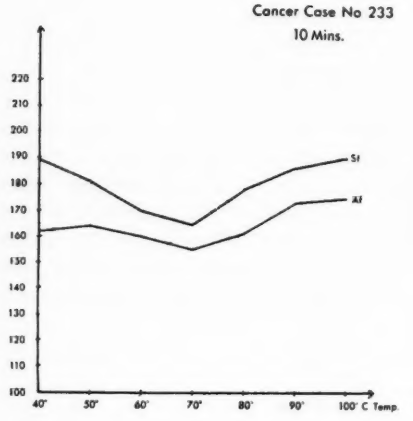

Fig. 23. Cancer case. Tests with denaturation for 10 mins. at different temperatures.

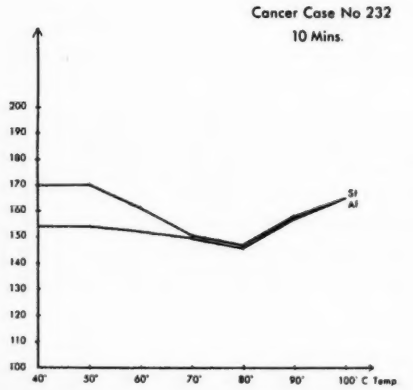

Fig. 24. Cancer case. Tests with denaturation for 10 mins. at different temperatures.

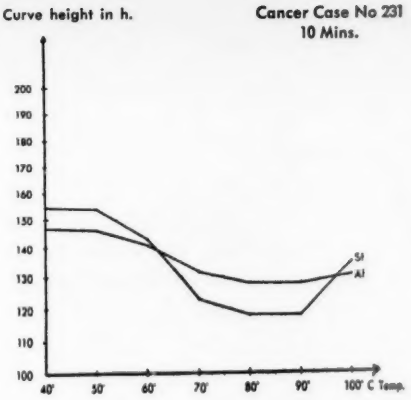

Fig. 25. Cancer case. Tests with denaturation for 10 mins. at different temperatures.

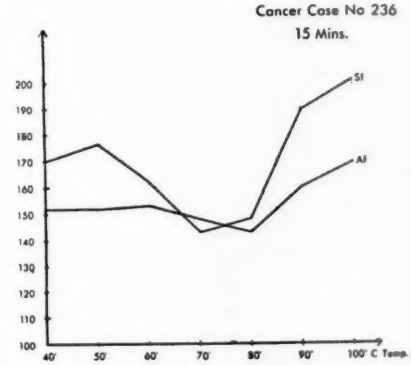

Fig. 26. Cancer case. Tests with denaturation for 15 mins. at different temperatures.

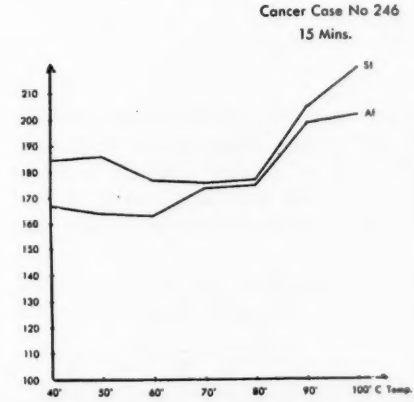

Fig. 27. Cancer case. Tests with denaturation for 15 mins. at different temperatures.

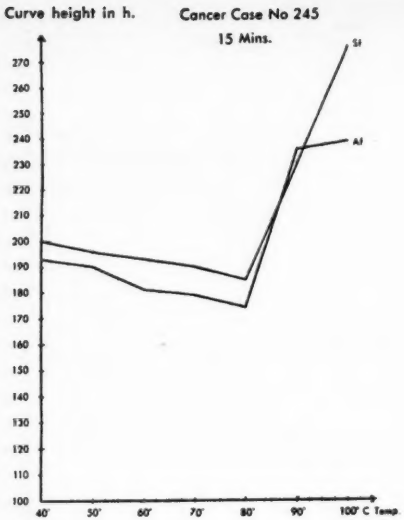

Fig. 28. Cancer case. Tests with denaturation for 15 mins. at different temperatures.

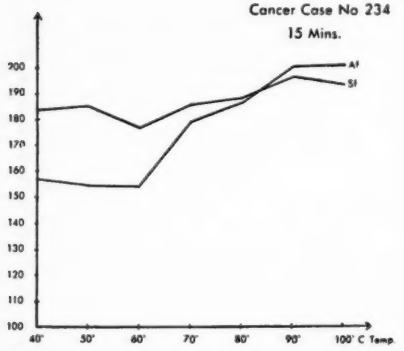

Fig. 29. Cancer case. Tests with denaturation for 15 mins. at different temperatures.

When stored in a refrigerator there was no change that exceeded the normal variation in the polarographic values in any single case, except for one cancer serum, where a slight increase could be observed in the values after it had been kept for 14 days. Neither was it possible to observe any difference betwen cancer and infectious sera.

A study of D shows that when infectious sera are kept at room temperature, there is a slight rise in D during the first 8 days, which

Fig. 30.

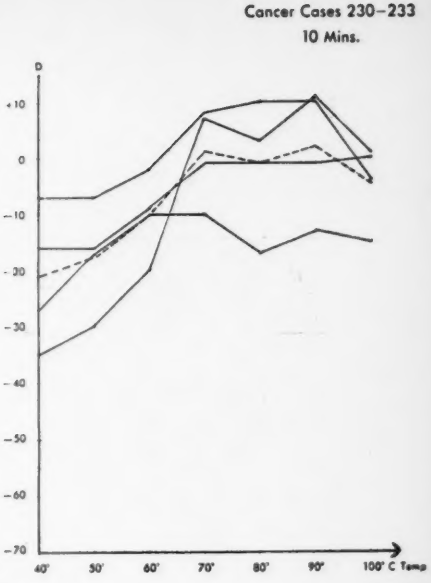

Fig. 31.

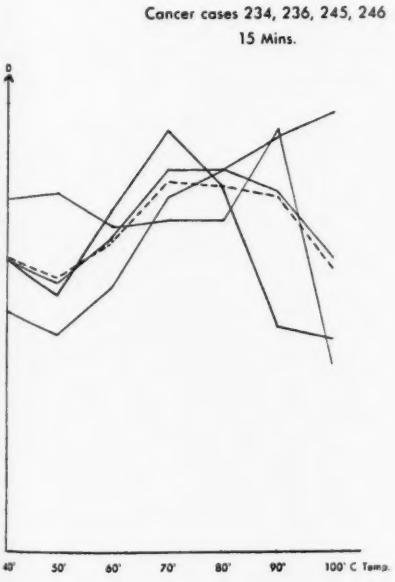

Fig. 32.

Fig. 30—32. D values for cancer cases. Tests with denaturation for 5—15 mins. at different temperatures. The dashed curves represent the mean values. (The curves correspond to figs. 19—21, 22—25 and 26—29 respectively.)

TABLE 19

Investigation on the Keeping Qualities of Cancer Sera when stored in a Refrigerator and at Room Temperature

a) Stored at room temperature (+ 20° — + 25° C)

No. of days and nights[1]	Sf					Af					D[2]
	88	113	87	89	90	88	113	87	89	90	
1	138	168	207	190	185	144	162	190	200	134	— 11.6
2	138	167	206	188	185	146	162	190	200	133	— 10.6
4	139	164	204	185	184	139	160	189	198	131	— 11.8
8	138	163	201	181	180	138	158	187	195	128	— 11.4
14	148	177	261	219	210	145	170	215	222	143	— 24.0

b) Stored in a refrigerator at + 4° C

No. of days and nights[1]	Sf					Af					D[2]
	88	113	87	89	90	88	113	87	89	90	
1	138	168	207	190	185	144	162	190	200	134	— 11.6
2	138	168	207	189	185	145	162	190	200	134	— 11.2
4	138	167	206	189	185	145	162	189	199	134	— 11.2
8	136	166	205	188	184	142	160	188	199	132	— 11.6
14	144	165	204	187	182	146	160	186	198	131	— 12 2

[1] "Number of days and nights" = number of days and nights between the taking of the blood sample and the analysis.

[2] The D values are calculated as the difference between the mean values.

TABLE 20

Investigation on the Keeping Qualities of Sera from Patients with infectious Diseases in a Refrigerator and at Room Temperature

a) Stored at room temperature (+ 20° — + 25° C)

No. of days and nights[1]	Sf					Af					D[2]
	730	731	732	733	734	730	731	732	733	734	
1	216	176	151	192	146	215	153	146	214	158	+ 1.0
2	214	175	149	192	144	215	152	145	213	157	+ 1.6
4	214	172	146	189	141	213	148	145	211	155	+ 2.0
8	210	169	147	186	139	212	146	145	210	153	+ 3.0
14	272	202	196	213	172	225	161	193	222	180	— 14.8

b) Stored in a refrigerator at + 4° C

No. of days and nights[1]	Sf					Af					D[2]
	730	731	732	733	734	730	731	732	733	734	
1	216	176	152	192	146	216	154	147	213	158	+ 1.2
2	216	176	152	193	146	215	154	147	214	158	+ 1.0
4	214	175	151	192	145	215	154	147	214	158	+ 2.2
8	214	174	152	192	144	215	153	147	213	157	+ 1.8
14	214	173	151	191	145	215	152	145	211	157	+ 1.2

[1] "Number of days and nights" = number of days and nights between the taking of the blood sample and the analysis.

[2] The D values are calculated as the difference between the mean values.

is due to the sulphosalicylic acid filtrate values sinking rather more quickly than those of the alcohol. When putrefaction sets in D sinks considerably owing to the values of sulphosalicylic acid filtrate increasing relatively more than those of the alcohol filtrate. A similar state is to be seen in the difference D, when cancer sera are kept at room temperature. The change in D is extremely slight, both in the case of cancerous and infectious sera when kept in a refrigerator. In the case of cancer sera kept at room temperature, D is practically constant up to 8 days. When putrefaction sets in D decreases considerably owing to the sulphosalicylic acid filtrate values increasing much more than those of the alcohol.

CHAPTER 12

Studies on the Effect of Variations in the Alcohol Concentration on Serum Polarograms in Normal and Pathological Sera

a) *Fractionation of Normal and Pathological Sera.*

As has already been mentioned in the introduction and in chapter 3, K. Mayer and E. Waldschmidt-Leitz considered that they were able to distinguish two fractions in the filtrate obtained after denaturation by heat and subsequent precipitation with sulphosalicylic acid of sera from cancer patients and from those with infectious or inflammatory diseases. Both writers indicated that fractionation of the filtrate with 66% alcohol produces a mucoid fraction and that by precipitation with 75—80% alcohol a fraction will be obtained containing protein products with an abundance of polarographically active disulphidic and sulphydryl groups.

In this section the writer intends to study the precipitation conditions of these fractions more closely and also their occurence in normal and pathological sera.

Applying the same method as in the previous section, 10 sera were investigated from healthy subjects, from cancer subjects and from those suffering from acute infectious diseases with fever. Each serum was fractioned by adding 1.83, 1.99, 2.16, 2.35, 2.56, 2.79, 3.05, 3.34, 3.67, 4.06 and 4.5 ml alcohol respectively to portions of 1.5 ml diluted serum denaturated for 12.5 minutes at 80° C. In the final mixture the alcohol concentration amounted to 55, 57, 59, 61, 63, 65, 67, 69, 71, 73 and 75% in the 11 portions from each serum. At every step in the fractionation two portions of serum were treated as indicated above, and one polarogram was taken of each one. The values given in tables 21—23 form the

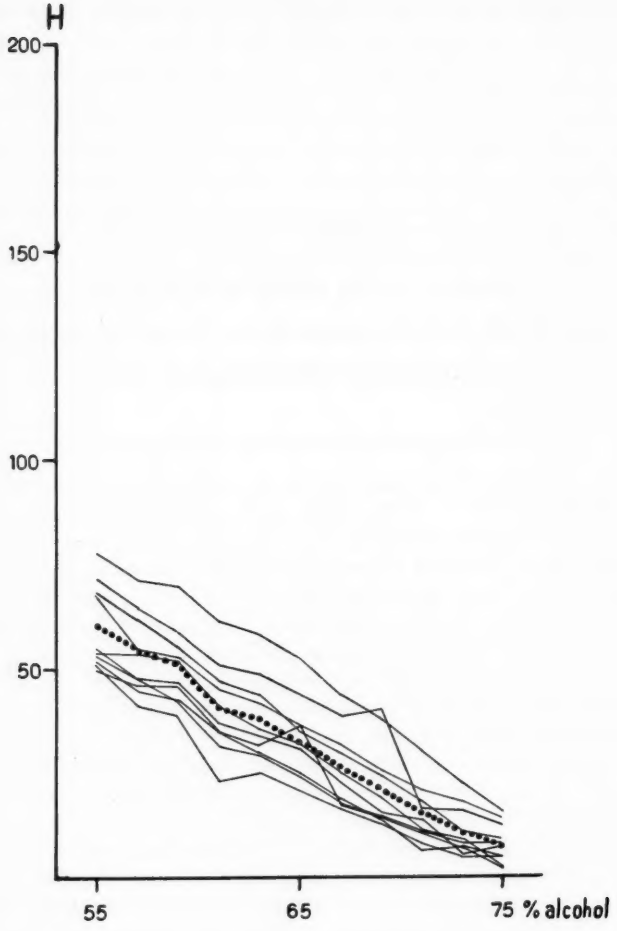

Fig. 33. H-values for normal cases. The curves correspond to the values in table 21. The dotted line represents the mean values.

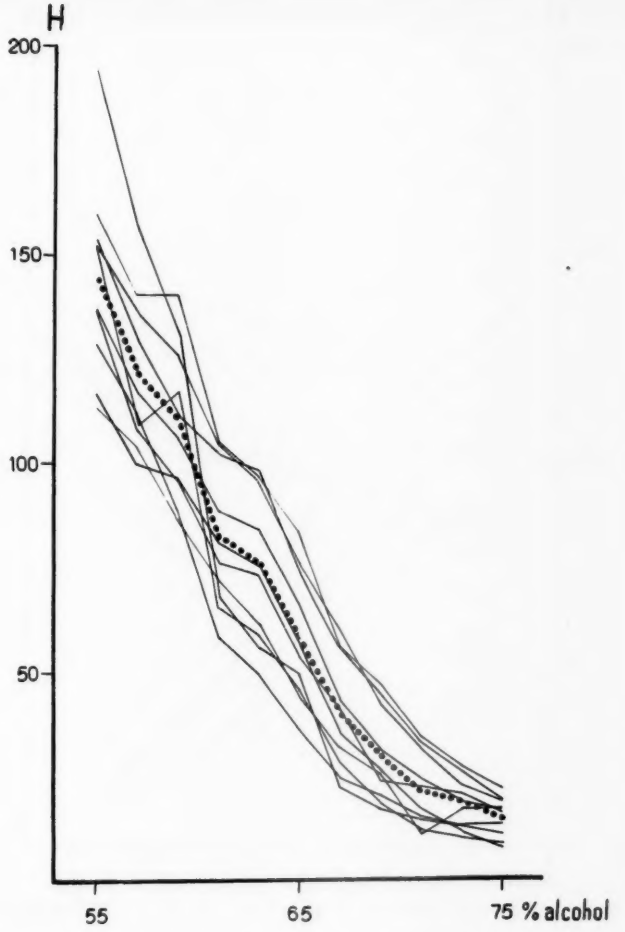

Fig. 34. H-values for cancer cases. The curves correspond to the values in table 22. The dotted line represents the mean values.

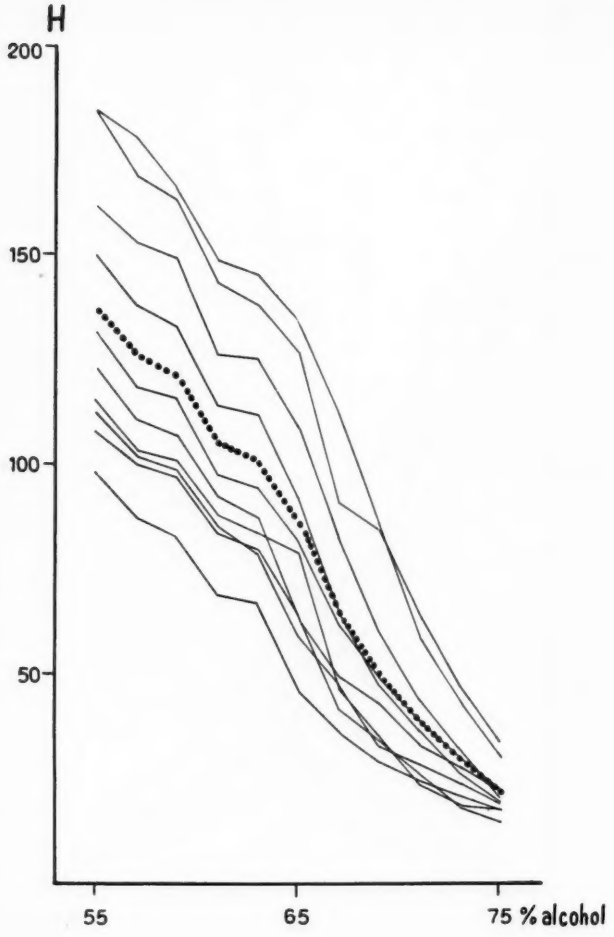

Fig. 35. H-values for infectious cases. The curves correspond to the values in table 23. The dotted line represents the mean values.

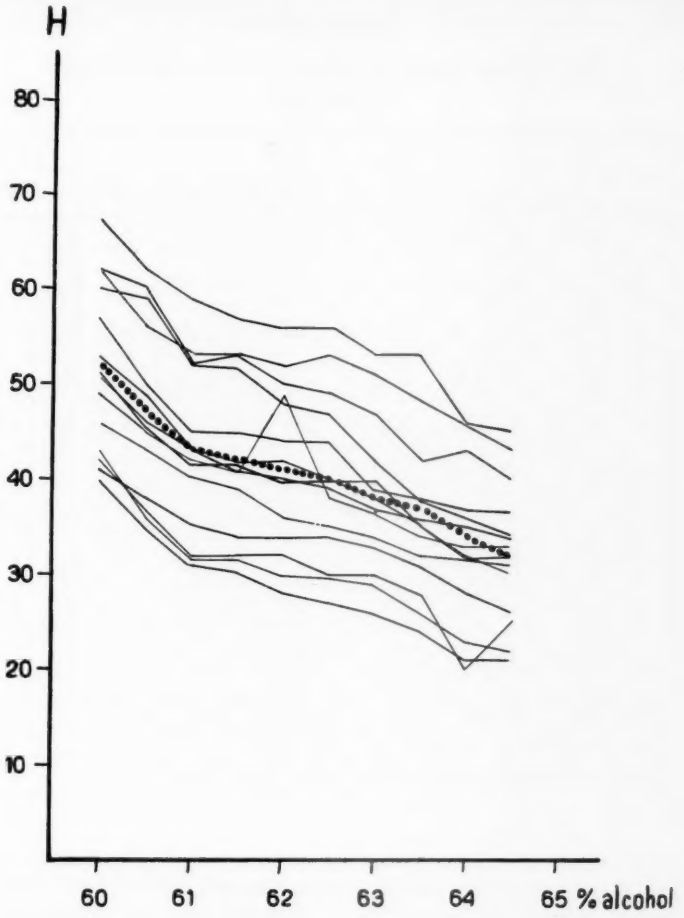

Fig. 36. H-values for normal cases. The curves correspond to the values in table 24. The dotted line represents the mean values.

TABLE 21

Alcohol filtrate values at different alcohol concentrations. Normal cases

Case	Sf-78	Af-78										
		55%	57%	59%	61%	63%	65%	67%	69%	71%	73%	75%
1	6	51	41	39	24	25	21	17	13	8	5	5
2	10	68	55	53	45	42	37	32	26	21	18	14
3	17	78	72	70	62	59	53	44	38	30	23	16
4	9	54	54	52	41	36	32	25	19	12	9	3
5	3	50	46	46	35	30	25	19	13	6	7	3
6	10	51	45	43	32	30	24	18	14	11	8	8
7	23	72	65	59	51	49	44	38	40	17	16	13
8	6	68	62	55	47	44	35	30	24	18	12	9
9	8	55	48	47	37	34	31	23	15	14	6	8
10	8	54	48	43	35	32	36	18	14	11	8	5
M	10	60.2	53.6	50.7	40.9	38.1	32.8	26.4	20.6	14.8	11.2	8.5

Sf-78 is carried out to show the values when serum is precipitated in the ordinary way with sulphosalicylic acid. M = mean value.

mean values of the heights of the curves in the two polarograms when the base value 78h has been subtracted. The last mentioned value corresponds to the height of the cobalt step, which is constant for all the analyses. The corresponding curves are to be found in figs. 33, 34 and 35.

In order to carry out a more thorough study of the fractionation curve within the region 61—63%, where a distinct plateau formation has made its appearance (see under c in this chapter), a corresponding fractionation was carried out on 14 normal sera with alcohol values of 60.0, 60.5, 61.0, 61.5, 62.0, 62.5, 63.0, 63.5, 64.0, 64.5%. The method and calculations of the values obtained are the same as those

TABLE 22

Alcohol filtrate values at different alcohol concentrations. Cancer cases

Case	Sf-78	Af-78										
		55%	57%	59%	61%	63%	65%	67%	69%	71%	73%	75%
1	107	152	109	117	66	59	45	28	18	12	10	9
2	75	117	100	97	76	73	54	41	24	23	20	16
3	82	160	140	140	105	97	83	56	47	34	27	22
4	108	152	136	126	105	97	76	60	42	32	23	19
5	75	137	108	97	81	75	56	35	26	17	11	9
6	83	129	111	89	59	50	36	25	20	16	14	14
7	88	114	104	87	72	62	45	32	25	12	17	17
8	74	136	117	106	88	84	66	44	31	24	19	17
9	108	153	130	112	102	97	74	56	45	33	27	19
10	114	195	157	131	68	56	49	22	17	14	14	11
M	91.4	144.5	121.2	110.2	82.2	75.0	58.4	39.9	29.5	21.7	18.2	15.3

Sf-78 is carried out to show the values when serum is precipitated in the ordinary way with sulphosalicylic acid. M = mean values.

just described. The values are to be found in table 24. The corresponding curves are to be found in fig. 36.

The cancer sera used came entirely from patients, in which it was possible to verify the diagnoses. The infection sera were selected from patients with acute infectious diseases at the febrile condition. All the cases are to be found in the brief casuistry.

b) *Calculation of a Correction Formula.*

When analysing the curves represented in tables 21—24, it is unsuitable to use the curve height values. If the results are to be

TABLE 23

Alcohol filtrate values at different alcohol concentrations. Infectious cases

Case	Sf-78	Af-78										
		55%	57%	59%	61%	63%	65%	67%	69%	71%	73%	75%
1	90	123	111	107	92	87	63	49	43	33	28	22
2	79	112	102	99	85	79	59	48	33	29	24	19
3	62	115	104	101	88	84	79	47	35	24	19	18
4	71	98	87	83	69	67	46	37	29	24	21	19
5	162	185	178	166	149	145	134	112	86	59	44	30
6	153	162	153	149	126	125	109	83	60	44	32	21
7	136	185	169	163	143	138	127	91	85	64	47	34
8	91	150	138	133	114	112	92	65	48	36	26	20
9	78	132	119	116	98	95	82	62	49	39	30	23
10	68	108	100	97	84	80	64	42	34	26	19	15
M	99.0	137.0	126.1	121.4	104.8	101.2	85.5	63.6	50.2	37.8	29.0	22.1

Sf-78 is carried out to show the values when serum is precipitated in the ordinary way with sulphosalicylic acid. M = mean values.

generally applicable, the heights of the curves should be converted into corresponding concentrations of polarographically active groups. The simplest way of doing this is by means of a calibration curve. The relation between the height of the protein wave (H) and this concentration follows the equation (see page 11)

$$H = \frac{a_1[\text{Prot}]}{1 + b[\text{Prot}]}$$

The absolute concentrations in the tests, however, are unknown. Instead one must content oneself with taking some given unknown

TABLE 24

Alcohol filtrate values at different alcohol concentrations. Normal cases, narrow region

Case	Sf-78	Af-78									
		60.0%	60.5%	61.0%	61.5%	62.0%	62.5%	63.0%	63.5%	64.0%	64.5%
432	6	42	37	32	32	32	30	30	28	20	25
433	30	67	62	59	57	56	56	53	53	46	45
444	10	41	38	35	34	34	34	33	31	28	26
445	17	53	49	43	41	40	39	37	36	35	34
446	17	57	50	45	45	44	44	39	38	37	37
447	17	51	46	43	42	42	40	38	36	32	30
448	6	40	35	31	30	28	27	26	24	21	21
449	12	46	43	40	39	36	35	34	32	32	31
450	2	43	36	32	32	30	30	29	26	23	22
451	13	49	45	42	41	49	38	37	34	33	33
452	26	62	56	53	53	52	53	51	48	46	43
453	18	60	59	52	52	48	47	42	38	36	34
454	18	51	45	42	42	40	40	40	35	32	32
455	23	62	60	52	53	50	49	47	42	43	40
M	15.4	51.7	47.2	43.0	42.4	40.8	40.1	38.3	37.1	33.6	32.4

Sf-78 is carried out to show the values when serum is precipitated in the ordinary way with sulphosalicylic acid. M = mean values.

concentration as a reference value. For this purpose the writer has chosen the concentration after precipitation with 62.5% alcohol.

The correction formula was determined in the following manner. After the initial 62.5% alcohol precipitation, the serum was diluted with water in a geometrical series. The volume of the sample was thus

doubled for each dilution. A polarographic analysis was carried out on each dilution.

In each dilution the concentration of proteins is decreased in the sample. Presuming that the contents of the polarographically active groups in the proteins do not undergo any change during the dilution, it is possible to calculate from the given dilution values the proportional change in the polarographic protein concentration, the concentration factor, cf. The formula will then be

$$H = \frac{a_1 \cdot cf \cdot [\text{Prot } 62.5\,\%]}{1 + b_1 \cdot cf\,[\text{Prot } 62.5\,\%]}$$

where $[\text{Prot}_{\,x\,\%}]$ signifies the concentration after precipitation with x% alcohol.

$$\text{Put } a_1\,[\text{Prot } 62.5\,\%] = a_2 \text{ and } b_1\,[\text{Prot } 62.5\,\%] = b_2$$

The formula can then be written as follows:

$$H = \frac{a_2 \cdot cf}{1 + b_2 \cdot cf}$$

or

$$\frac{H}{cf} = a_2 - b_2 \cdot H$$

Practically the correction formula was determined in the following manner. Polarograms were taken from three different filtrates, each constituting alcohol filtrates, which were mixed together, were of equal size and taken from 20 patients. The dilutions and polarographic analyses were carried out as mentioned above. From the values obtained the correction formula was calculated as a regression line with $\frac{H}{cf}$ and H as the variables.

The H-values are expressed in h units.

As has already been mentioned above, when determining the calibration curve the concentration after a 62.5% alcohol precipitation was taken as the reference concentration ($cf = 1$).

The adaptation of the separate values to the H values of the regression line was good for H $>$ 20h and somewhat worse for the lower H values. The figure 20h corresponds to a cf value of 0.17.

The correction formula will be

$$\frac{H}{cf} = 142.38 - 1.02 \cdot H$$

or

$$cf = \frac{0.98 \cdot H}{139.6 - H} = \text{approx.} \frac{H}{140 - H}$$

It is perfectly natural, however, that this concentration must vary in different individuals, though to what extent is not known. Thus an error is made when adapting the calibration curve to the particular case. In order to obtain an "average" calibration curve the 60 test subjects were taken from the author's medical practice completely at random. The material comprises 36 patients, who may be considered "healthy", 11 with cancer and 13 with acute infectious diseases with fever. The material was chosen in this manner, since the curves to be converted come from both healthy and sick subjects.

However, "the concentration values" obtained with the calibration curve represent only the concentrations in the final mixture of cobalt solution and filtrate. When converted into corresponding concentration factor for the original, undiluted serum tests, the curves will assume a somewhat different form.

If the concentration in the filtrate is kf and in the serum k_s, it will easily be realised that

$$k_s = \frac{(1 + 0.5)}{0.5} \cdot \frac{(5 + 0.5) \cdot (1.5 + a)}{0.5 \cdot (1 + 0.5)} \cdot kf = 22\ (1.5 + a)\ kf$$

since during the analysis 0.5 ml serum was diluted with 1 ml aq. redistillata to (1+0.5) ml. This quantity was denaturated by heat and then precipitated with "a" ml alcohol, after which 0.5 ml of the filtrate was mixed with 5 ml buffer solution (0.5+5.0), and analyzed polarographically.

The concentration after a 62.5% alcohol precipitation was chosen as the unit for the calculations of the concentration in the filtrate: [Prot $_{62.5\ \text{filtrate}}$]. The corresponding unit for the serum will then be

$$[\text{Prot}_{62.5\ \text{serum}}] = 88 \cdot [\text{Prot}_{62.5\ \text{filtrate}}]$$

since here a=2.5 ml.

If cf is converted to the corresponding serum factor c_s with [Prot 62.5 serum] as reference concentration, it will give

$$c_s = \frac{1.5 + a}{1.5 + 2.5} \cdot cf = \frac{1.5 + a}{4} \cdot cf$$

In these calculations it was assumed that the effective volume for the concentration of the polarographically active substances is the same as the total volume given, i. e. that the change in the proportion of alcohol:H_2O has no influence on the effective volume.

This assumption holds good for ordinary salts (cobalthexammine-chloride) (table 25). As regards proteins in the low concentrations dealt with in this work the relation is not known, though it may be possible that the protein acts in the same way as the salt.

TABLE 25

Analysis with the same method as in tables 21—24 but with water instead of serum

Test no	Alcohol concentration										
	55%	57%	59%	61%	63%	65%	67%	69%	71%	73%	75%
1	78	78.5	78	77	78	77.5	79	78	78	78	78
2	77,5	78	78	78	77	78	78	78	77	78.5	78.5
3	78	78	77.5	78	79	78	76.5	78	77	78	79
Mean value	78	78	78	78	78	78	78	78	77	78	78

c) *The C_s-curves for the three Kinds of Sera.*

In the diagrams 37—41 the curves for the normal and pathological cases in figs 33—36 have been converted to c_s values according to the above. The figures will give an approximate picture of the actual relations between the three protein fractionation curves.

When scrutinizing the curves we are struck by certain regularities and parallels between the separate curves. In the interval of 59—63% alcohol concentration it will be seen that all the curves without exception "bulge downwards". Such agreement cannot en-

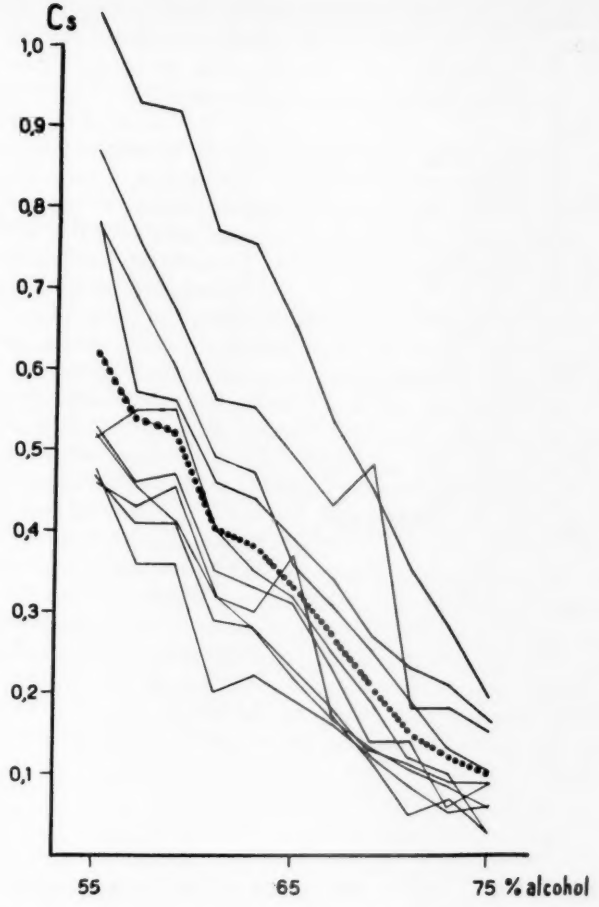

Fig. 37. C_s-values for normal cases. The dotted line represents the mean values.

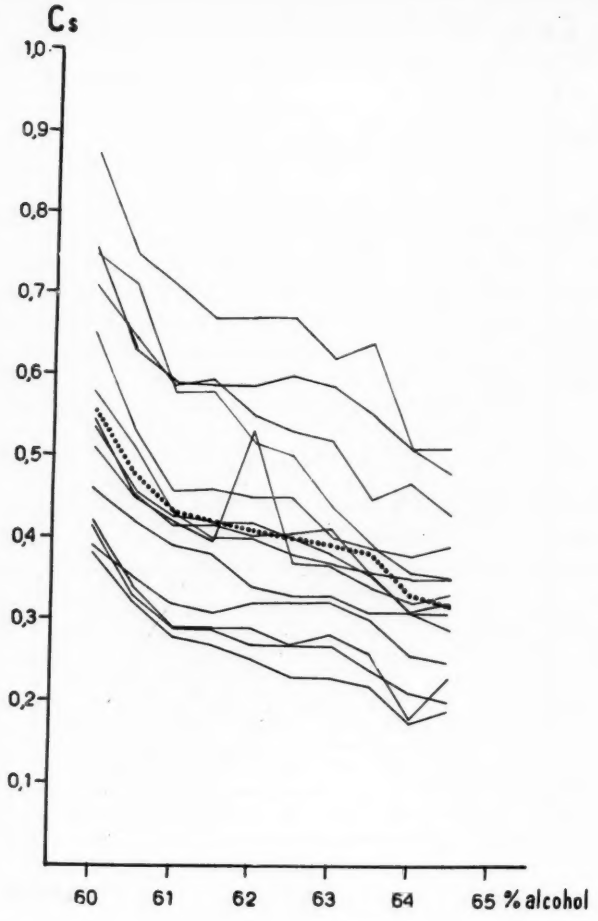

Fig. 38. C_s-values for normal cases, narrow region. The dotted line represents the mean values.

tirely be due to chance. If the values of the curves showed simply a random deviation a number of the bulges could just as well go upwards within the same interval. The probability is exceedingly small $(\frac{1}{2})^{30}$ for this part of the curve to bulge downwards at random in all the 30 cases.

An explanation of the bulge in the curve comes perfectly natural, for if after a 55% alcohol precipitation there still remain protein substances of various characters in the sera, there should be an ordinary fractionation curve with steps. As it is a question of such small absolute quantities of protein in this case, the curves will not, however, be so indisputably clear.

Thus it seems as though the filtrates investigated contain 3 different protein fractions varying both in quantity and quality, firstly the one found in the alcohol concentration interval 59—63 %, and secondly those above and below this alcohol concentration.

The investigation of the intervals between the 60.0 and the 64.5% alcohol concentration with 0.5% steps (fig. 38), likewise show how the curves within this interval bulge downwards. As before the curves represent the factor c_s. They can thus be compared with the other diagrams and show the same tendency.

The calculations made in this chapter are extremely approximate, and as already pointed out, are influenced by several sources of error. Nevertheless they aim at giving a picture of the changes in concentration to be found behind the recordings of the polarograph. One gets the impression that one has to do with three distinct protein fractions differing in quantity or quality, and that these fractions are always distinctly marked in the individual fractionation curves.

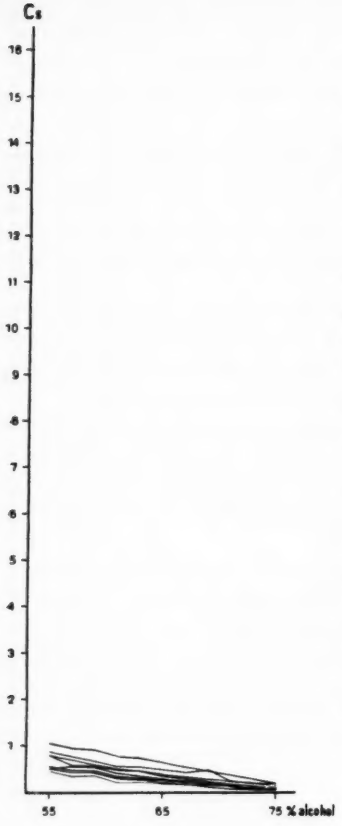

Fig. 39. C_5-values for normal sera. The same diagram as Fig. 37 but drawn in another scale.

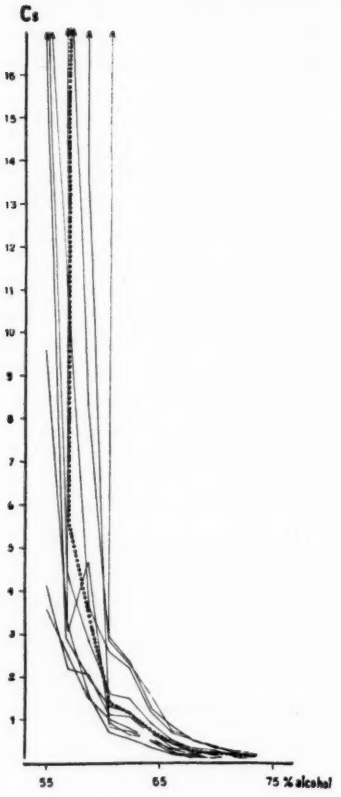

Fig. 40. C_5-values for cancer sera. The dotted line represents the mean values.

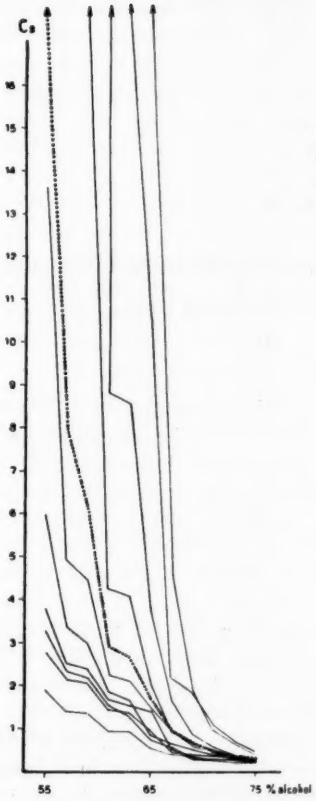

Fig. 41. C_5-values for infectious sera. The dotted line represents the mean values.

Fig. 39—41. The C_5-values for the three kinds of sera. In the same reproduction scale.

CHAPTER 13

Variations in the Relations between Filtrate Fractions. Normal, Cancer and Infectious Sera.

The occurence of variations in the relation between the concentrations of various proteins in blood serum in different pathological states is too well known for it to be necessary to go into in detail in this work. As is the case of the protein substances in the blood with high molecular weights, varying concentrations may be expected in the small absolute quantities of the proteins with low molecular weights or decomposition products of proteins registered in the polarographic filtrate analysis.

As has already been briefly mentioned in chapter 3, K. Mayer and E. Waldschmidt-Leitz (92, 153, 156) found that when sera, denaturated by heat and precipitated with 25% sulphosalicylic acid, are fractionated by alcohol, they obtained two fractions with precipitation qualities that are in close agreement with what the writer of this work has discovered. K. Mayer also attempted to investigate in greater detail the chemical composition of the fractions by means of purification and chemical analysis. They then came to the conclusion that by precipitation with 66% alcohol a fraction could be obtained consisting of a mucoid-like substance, mixed with small quantities of proteins of low molecular weight. The second fraction obtained by precipitation with 90% alcohol consisted of protein products containing disulphidic and sulphydryl groups. Whether purer products can be obtaned by precipitation with 61—63% (62%) alcohol, which according to the writer's investigations seems to be more favourable, remains to be proved.

Based on the opinion that the mucoid-like fraction originates from cancer cells in the organism, while the other appears (or is found

in increased quantities) in infectious and inflammatory diseases, Waldschmidt-Leitz and Mayer worked out a diagnostic method with the object of improving Brdicka's reaction with regard to the possibility of making a differential diagnosis between cancer and the latter pathological states. Both these writers as well as others having tried the method, inter alia Merten (99), found that like Brdicka's reaction, it gave a positive result for the majority of cancer cases and a varying number of infectious and inflammatory states. On the other hand it was not possible to notice any definite improvement in relation to the Brdicka reaction (considered as a cancer reaction) as regards the possibility of making a differential diagnosis between cancer in one case and other pathological states in the other.

The investigations carried out by the author can also form the basis of a study as to how the relations between the curve heights of the fractions above and below the 61—63% alcohol concentration vary in normal, cancer and infectious sera.

Since these measurements are not intended to express the true quantities of protein corresponding to the height of the polarographic curve but only to some suitable, quantitative units, they may conveniently be performed from the values of the heights of the curves. The latter will also be the most suitable for this purpose, as they are only impaired by a relatively small error of method and not by the comparatively rough approximations that the tests in connection with the measurements of the actual SH concentration in serum compel.

The fractions were measured according to figs. 42—44. In these figures there will be found the mean curves (the H-curves) for the three kinds of sera. The measurement of the most soluble fraction, which is the nearest approach to K. Mayer's and E. Waldschmidt-Leitz' sulphur-containing one and which in this work is termed f_2, offers no difficulties. The "blank value" of 78h is chosen as the "base" value. The centre point in the "step" formed at the 61—63% alcohol concentration was selected as the second one, i. e. at the 62%, the change over between the fractions apparently being somewhere between 61 and 63%.

The base value when measuring the less soluble fraction here termed f_1, and the nearest approach to the mucoid-like fraction of the writers just mentioned, will be the height of the curve at the 62% alcohol concentration. The upper limit of this fraction seems to be somewhere between the 57 and the 59% alcohol concentration,

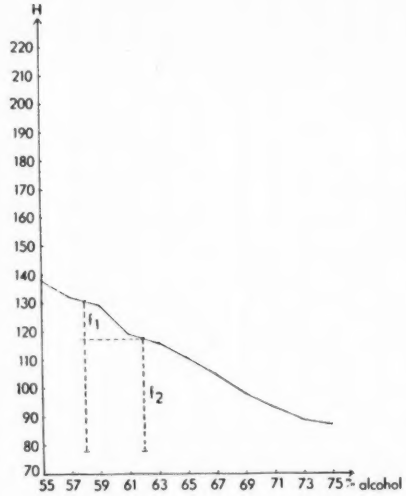

Fig. 42. f_1 and f_2 for normal cases. The curve represents the mean H-values for the 10 cases in table 21.

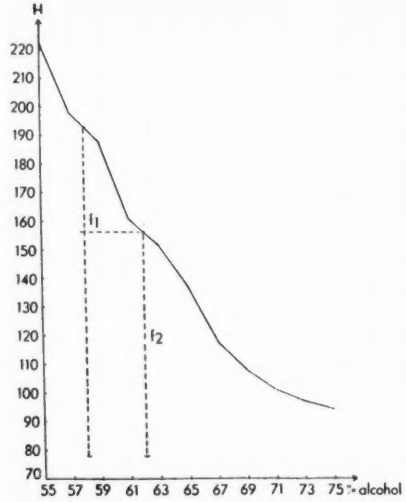

Fig. 43. f_1 and f_2 for cancer cases. The curve represents the mean H-values for the 10 cases in table 22.

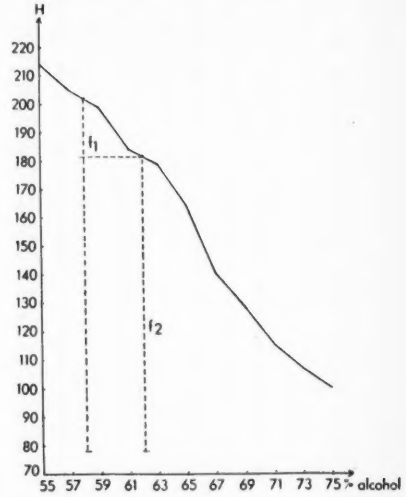

Fig. 44. f_1 and f_2 for infectious cases. The curve represents the mean H-values for the 10 cases in table 23.

where there is another distinct plateau formation, approximately 58% alcohol.

The value of f_1 and f_2 as well as the ratio of $\frac{f_2}{f_1}$ are to be found in table 27.

From the calculations it will be seen that f_1+f_2 in the case of normal sera is on an average lowest, in the case of infection sera it is on an average highest and that cancer sera has a value between these two but closer to the infection sera. In the case of the latter the f_2 fraction is greater than that of cancer sera, whereas the f_1 fraction is lowest in infection sera and highest in cancer.

One gets a definite impression that there occur changes of a quantitative nature—the sera containing both fractions though in varying quantities.

The statistical analysis is given in table 26. The differences between the means from table 27 are tested by use of an ordinary t-test.

It would seem that the absolute values of the f_1 and f_2 fractions increase in all the pathological sera as compared with the normal.

On the other hand the relative increase of the f_2 and f_1 fractions seems to differ as regards cancer and infectious sera.

This investigation may perhaps seem to be unreliable in view of the small groups of inviduals tested. On the other hand the result seems upon the whole to corroborate the generally accepted ideas of these pathological events.

In the cases of cancer we probably have an increased quantity of protein decomposition products from the tumour in the blood. These low molecular products are liberated by the heat denaturation of the serum. Thus the two fractions would be increased as compared with the quantity in normal sera. But on the other hand they do not necessarily have to be changed as regards their relative quantities.

In the same way both fractions seem to be increased in infectious sera. But here the results indicate that the relative quantity may differ from the normal. This can have been brought about in many different ways: it can be a mixture of decomposition products from the infectious foci, perhaps with an admixture of bacterial protein. It can also depend upon the mechanism of immunity (where especially the SH-groups are active).

Thus the quantitative background to the polarographic curves as regards the protein concentration is uncertain. Nevertheless it is obvious that there must be some sort of discontinuity behind the "fractionation curves" found, whether it be in the protein quantities

TABLE 26

Statistical analysis of the f_1 and f_2 values

Difference between	f_2	f_1	$f_2 : f_1$
infectious — normal (10 cases) (10 cases)	63.5 ± 9.1 7.0 0.001 stat. sign.	8.2 ± 1.3 6.3 0.001 stat. sign.	1.67 ± 0.42 4.0 0.001 stat. sign.
cancer — normal (10 cases) (10 cases)	39.1 ± 6.4 6.1 0.001 stat. sign.	25.0 ± 5.8 4.3 0.001 stat. sign.	— 0.70 ± 0.55 1.3 0.2 not sign.
infectious — cancer (10 cases) (10 cases)	24.4 ± 10.1 2.4 0.03 prob. sign.	— 16.8 ± 5.9 2.8 0.01 prob. sign.	2.37 ± 0.43 5.5 0.001 stat. sign.
The values are	difference of means ± error of difference t-value p = probability that the difference may arise at random. significance		

precipitated, changes in the number of SH groups in the proteins or in both.

The sera used in the investigations all originate from advanced cases of the respective diseases. As it seems probable that the variations are more or less parallel quantitatively with the severity and the development of the disease (39), the difference will probably be less pronounced at the outset and recovery respectively than is the case here.

Bearing in mind the attempts of other writers to make clinical use of these conditions, the writer feels that he should sum up his own standpoint in the following manner: The two fractions in the filtrate established for the three kinds of sera show in themselves some variations in quantities proving significant in a statistical analysis of this material.

A closer study of the qualities, the chemical nature and origin of the substances as well as their appearance and relation during the genesis, course and recovery of various pathological states, might perhaps contribute to a further penetration into the protein metabolism in the organism, particularly as the polarographic serum reaction is based on the physiologically important sulphydryl and disulphidic groups. Only a thorough study of the problem can give an answer to the question as to the value of the phenomena observed from a clinical viewpoint.

TABLE 27

f_1 and f_2 values. Relation f_2+f_1 and $f_2:f_1$

	f_2	f_1	$f_2 + f_1$	$f_2 : f_1$
Cancer cases	62.5	50.5	113.0	1.24
	74.5	24.0	98.5	3.10
	101.0	39.0	140.0	2.59
	101.0	30.0	131.0	3.37
	78.0	24.5	102.5	3.18
	54.5	45.5	100.0	1.20
	67.0	33.5	100.5	2.00
	86.0	25.5	111.5	3.37
	99.5	21.5	121.0	4.63
	62.0	82.0	144.0	0.76
Mean ± error of mean	78.6 ± 5.5	37.6 ± 5.8	116.2 ± 5.4	2.54 ± 0.39
Stand. dev.	17.5	18.4	17.1	1.22
Infectious cases	89.5	19.5	109.0	4.59
	82.0	18.5	100.5	4.43
	86.0	16.5	102.5	5.21
	68.0	17.0	85.0	4.00
	147.0	25.0	172.0	5.88
	125.5	25.5	151.0	4.92
	140.5	25.5	166.0	5.51
	113.0	22.5	135.5	5.02
	96.5	21.0	117.5	4.60
	82.0	16.5	98.5	4.97
Mean ± error of mean	103.0 ± 8.5	20.8 ± 1.2	123.8 ± 9.7	4.91 ± 0.17
Stand. dev.	27.0	3.7	30.5	0.54
Normal cases	24.5	15.5	40.0	1.58
	43.5	10.5	54.0	4.14
	60.5	10.5	71.0	5.76
	38.5	14.5	53.0	2.66
	32.5	13.5	46.0	2.41
	31.0	13.0	44.0	2.38
	50.0	12.0	62.0	4.17
	45.5	13.0	58.5	3.50
	35.5	12.0	47.5	2.96
	33.5	12.0	45.5	2.79
Mean ± error of mean	39.5 ± 3.3	12.6 ± 0.5	52.2 ± 3.0	3.24 ± 0.38
Stand. dev.	10.1	1.6	9.5	1.20

PART IV

The Polarographic Serum Reaction During some Physiological Processes

CHAPTER 14

Studies on the Polarographic Serum Reaction During the Resorption of Foodstuffs

In order to study whether the serum reaction is affected by the resorption of foodstuffs, a series of analyses was carried out on healthy subjects, who had to eat food consisting chiefly of carbohydrates, proteins or fats.

The method was the same as described in previous chapters. The analysis was carried out with double tests of both sulphosalicylic acid and alcohol filtrates as well as with double polarograms of each filtrate.

Table 28 gives the result of the investigation of the effect produced on the serum reaction by food rich in carbohydrates. Blood tests were taken on empty stomachs before the investigation was begun. Each test subject was then given 150 gm crisp bread, 100 gm sugar and 100 gm milk. After the completion of the meal blood tests were taken for polarographic analysis, 2, 4 and 6 hours after the meal, respectively. In all the four tests values were obtained showing that no statistically definite changes had appeared.

Table 29 gives the result of investigations on the effect produced on the serum reaction by food rich in proteins. Each test subject had to eat 200 gm boiled beef and 2 eggs boiled soft. Blood tests were taken on empty stomachs before the test was begun, and 2, 4

TABLE 28

Investigation on the Effect of Carbohydrate on the Serum Polarograms

Case, food, etc.	Time of Test	Sf	Af	D
S. Ö. Woman. Age 34. Weight 65 kg. 150 gm crisp bread + 100 gm sugar + 100 gm milk	Empty stomach	98	118	20
	2 hrs. after meal	98	118	20
	4 hrs. after meal	99	120	21
	6 hrs. after meal	99	120	21
K. B. Woman. Age 35. Weight 63 kg. 150 gm crisp bread + 100 gm sugar + 100 gm milk	Empty stomach	116	142	26
	2 hrs. after meal	116	141	25
	4 hrs. after meal	117	142	25
	6 hrs. after meal	116	141	25
T. P. Woman. Age 31. Weight 58 kg. 150 gm crisp bread + 100 gm sugar + 100 gm milk	Empty stomach	92	117	25
	2 hrs. after meal	93	117	24
	4 hrs. after meal	93	117	24
	6 hrs. after meal	93	117	24
A. F. Woman. Age 26. Weight 55 kg. 150 gm crisp bread + 100 gm sugar + 100 gm milk	Empty stomach	108	133	25
	2 hrs. after meal	108	133	25
	4 hrs. after meal	108	132	24
	6 hrs. after meal	108	132	24

and 6 hours after the completion of the meal. The serum polarograms of these four test subjects do not show any statistically definite changes in this test either.

Finally, table 30 shows the result from corresponding tests carried out with food rich in fats. Each test subject ate 150 gm butter. From the table it will be seen that there are no statistically definite changes in the polarograms of the four tests.

TABLE 29

Investigation on the Effect of Protein on the Serum Polarograms

Case, food, etc.	Time of Test	Sf	Af	D
B. S. Woman. Age 20. Weight 59 kg. 200 gm beef + 2 boiled eggs	Empty stomach	103	126	23
	2 hrs. after meal	101	128	27
	4 hrs. after meal	102	125	23
	6 hrs. after meal	100	124	24
A. H. Woman. Age 64. Weight 74 kg. 200 gm beef + 2 boiled eggs	Empty stomach	107	128	21
	2 hrs. after meal	106	127	21
	4 hrs. after meal	106	126	20
	6 hrs. after meal	105	126	21
S. Ö. Woman. Age 34. Weight 65 kg. 200 gm beef + 2 boiled eggs	Empty stomach	104	124	20
	2 hrs. after meal	103	125	22
	4 hrs. after meal	106	126	20
	6 hrs. after meal	104	125	21
T. P. Woman. Age 26. Weight 58 kg. 200 gm beef + 2 boiled eggs	Empty stomach	92	117	25
	2 hrs. after meal	92	117	25
	4 hrs. after meal	93	117	24
	6 hrs. after meal	93	118	25

TABLE 30

Investigation on the Effect of Fat on the Serum Polarograms

Case, food, etc.	Time of Test	Sf	Af	D
M. N. Man. Age 39. Weight 75 kg. 150 gm butter on empty stomach	Empty stomach	106	128	22
	2 hrs. after meal	107	129	22
	4 hrs. after meal	107	129	22
	6 hrs. after meal	109	130	21
F. B. Woman. Age 35. Weight 63 kg. 150 gm butter on empty stomach	Empty stomach	130	152	22
	2 hrs. after meal	130	151	21
	4 hrs. after meal	130	151	21
	6 hrs. after meal	130	152	22
T. P. Woman. Age 26. Weight 58 kg. 150 gm butter on empty stomach	Empty stomach	92	117	25
	2 hrs. after meal	92	118	26
	4 hrs. after meal	93	118	25
	6 hrs. after meal	93	118	25
A. F. Woman. Age 26. Weight 55 kg. 150 gm butter on empty stomach	Empty stomach	109	132	23
	2 hrs. after meal	109	133	24
	4 hrs. after meal	109	132	23
	6 hrs. after meal	109	133	24

CHAPTER 15

Studies on the Polarographic Serum Reaction in Rest and Work

The question as to wether the increasing metabolism taking place in the body during physical work causes a rise of polarographically active decomposition products of protein visible in the filtrate reaction, has been made the subject of an elucidatory investigation.

An examination was carried out on each of altogether 6 persons, who had done hard physical work. Samples were taken, firstly before work began in the morning, secondly during and after work was over. In two cases samples were also taken 3½ hours after the work was finished, and the test subject had been resting. The results of the analyses will be found in table 31.

In the cases of A.F. and W-r, tests were taken on empty stomachs and while the test subjects were still resting as well as after 3 hours' quick ski-ing in terrain. Control tests were also taken 3½ hours after the ski-ing was over. In both cases the Sf and the Af showed rises in the values after the work was done, while after 3 1/2 hours' rest the values had returned to the original. A rise of this magnitude cannot have occurred by mere chance (cp. error of method).

In the cases of P-d and E-man the work consisted in digging earth in three different periods, the first being from 7—9 a.m., the second, 10—1 p.m. and the third from 1.30—4 p.m. The tests were taken at 7 a.m. before the work was begun and then after the end of each period. Both cases showed that the Sf and the Af values rose after work.

In the cases of E.W. and O.J. the work was the same as for the two previous test subjects. The work was carried out from 7—9 a.m.

TABLE 31

Values obtained from samples taken before, during and after physical work

	Time	Sf	Af	D
A.F., woman, age 21. SR: 13 mm. Test 11 a.m. on empty stomach ..	11.00 a.m.	98	122	24
Test 3.30 p.m. after ski-ing for 3 hrs. in terrain	3.30 p.m.	102	128	26
Test 7 p.m. after resting for 3 1/2 hrs.	7.00 p.m.	96	122	26
W-r., man, age 23. SR: 1 mm. .. Test 11 a.m. on empty stomach ..	11.00 a.m.	110	131	21
Test 3.30 p.m. after ski-ing for 3 hrs. in terrain	3.30 p.m.	114	134	20
Test 7 p.m. after resting for 3 1/2 hrs.	7.00 p.m.	110	131	21
P-d., man, age 37. SR: 4 mm.	7.00 a.m.	114	138	24
Test at 7 a.m. on empty stomach before beginning work	9.00 a.m.	117	140	23
Work with excavating earth 7—9 .. a.m., 10—1 p.m. and from	1.00 p.m.	116	138	22
1.30—4 p.m.	4.00 p.m.	119	141	22
E-man, man, age 42. SR: 7 mm. ..	7.00 a.m.	134	141	7
Test at 7 a.m. on empty stomach before beginning work	9.00 a.m.	138	144	6
Work with excavating earth 7—9 .. a.m., 10—1 p.m. and from	1.00 p.m.	138	145	7
1.30—4 p.m.	4.00 p.m.	140	149	9
E. W., man, age 61. SR: 8 mm. ..	7.00 a.m.	163	179	16
Test at 7 a.m. on empty stomach before beginning work	9.00 a.m.	166	182	16
Work with excavating earth 7—9 .. a.m. and from 10—1 p.m.	1.00 p.m.	167	183	16
O. J., man, age 62. SR: 19 mm. ...	7.00 a.m.	156	164	8
Test at 7 a.m. on empty stomach before beginning work	9.00 a.m.	162	171	9
Work with excavating earth 7—9.. a.m. and 10—1 p.m.	1.00 p.m.	163	172	9

SR = Sedimentation rate of red blood cells in mm/hour according to Westergren's method.

and from 10 a.m.—1 p.m. Tests were taken firstly before the work began, and secondly after the end of each period. These two cases again showed rising values after work.

The investigation also shows that the increase in the height of the curves seems to a certain extent to be correlated to the intensity of the work and possibly also to the duration of the work.

The tests show that physical work produces an increase in the polarographically active substances in the filtrate precipitated with sulphosalicylic acid and alcohol respectively. In view of what is already known concerning the chemical nature of these substances, it may be presumed that they originate from an increase in the decomposition of protein in the body during physical work.

It may be of interest also to notice that there are no definite changes in D during physical work.

CHAPTER 16

Studies on the Effect of Menstruation and Pregnancy on the Polarographic Serum Reaction

In order to study how the polarographic serum-reaction is affected by the various phases in the menstrual cycle, an examination has been carried out on four healthy women, their ages being 34, 22, 20 and 20 respectively. The blood samples were taken at intervals of a few days. The polarographic values obtained will be found in table 32. The dates when the tests were taken will be seen in the diagrams in figs. 45, 46, 47 and 48.

A study of the curves obtained shows that they all present the same basic type. A considerable decline in the polarographic values about 5—8 days and nights previous to the start of the menstruation, with the minimum value about 24—48 hours before the last mentioned point of time, is followed by a rapid increase in the height of the curves. This seems to be most strongly pronounced during the first 2—3 days, the curve reaching its highest value about 3—4 days after menstruation has begun. Afterwards there is another decline in the values, though this is not so pronounced as the one first mentioned. The decline reaches its minimum value after about 3—5 days. A slow rise then sets in and continues till the maximum is reached at a point of time, which seems to be just between the periods. During the latter half of the intermenstrual period the values again begin to decline slowly, again to undergo a rapid drop about 5—8 days before the next menstruation. Broadly speaking both the sulphosalicylic acid filtrate and the alcohol filtrate reactions seem to undergo the same changes. However, if we study the variations in D (the dashed curves), it will be seen that this difference also seems to change. In the four tests carried out, the

TABLE 32

Values obtained from samples taken during menstrual cycles in healthy women. (For dates see figs. 45—48).

S. Ö. Age 34			B. S. Age 20			K. C. Age 20			A. F. Age 22		
Sf	Af	D	Sf	Af	D	Sf	Af	D	Sf	Af	D
103	124	21	103	128	25	108	137	29	106	130	24
103	123	20	102	130	28	102	132	30	105	128	23
103	124	21	99	128	29	111	136	25	106	130	24
102	122	20	95	122	27	116	144	28	108	131	23
93	116	23	93	119	26	108	130	22	108	131	23
91	113	22	91	117	26	104	128	24	107	128	21
100	121	21	97	128	31	105	129	24	106	128	22
96	117	21	100	124	24	107	130	23	106	127	21
96	116	20	96	123	27	107	130	23	101	122	21
98	118	20	95	123	28	108	132	24	97	117	20
99	120	21	99	124	25	110	132	22	111	132	21
100	120	20	101	126	25	110	132	22	109	129	20
97	119	22	103	126	23	109	132	23	104	125	21
94	118	24	103	127	24	110	132	22	104	127	23
92	113	21	102	126	24	101	121	20	106	126	20
100	121	21	98	121	23	108	138	30	107	127	20
103	123	20	103	129	26	120	141	21	108	127	19
			105	125	20	114	135	21	109	129	20
			102	126	24						

test subjects S. Ö. and K. C. show an increase in D simultaneously with the pronounced decline in the Sf—Af values at the beginning of the menstruation.

In the case of A. F. the curves do not allow any definite conclusion as regards D. In the case of B. S. a rise in D sets in during the first menstruation period observed, which rise, broadly speaking, coincides with the decline of Sf and Af, which sets in just before menstruation begins. In addition there is a pronounced increase in D corresponding to the next ensuing rise in Sf and Af during the menstruation then going on. In the case of the second menstruation period observed there is a less pronounced rise of D, corresponding approximately to the premenstrual, marked decline of Sf and Af.

From the examinations undertaken it seems that both Sf and Af undergo regularly recurring variations during the menstrual cycles. These changes may be summarized in the following manner:

1. There is a pronounced decline in the filtrate values 5—8 days previous to the menstruation. The minimum is reached 1—2 days before the beginning of the menses.
2. There is a considerable increase in the values, commencing about 24 hours before menstruation begins. The increase is most strongly pronounced during the first 2—3 days. The maximum is reached 3—4 days after menstruation has begun.
3. A less pronounced decline during the subsequent 3—4 days.
4. A slow increase in the values, which continues till a point of time just between the menses.
5. During the latter half of the intermenstrual phase at first slowly declining values, which drop more quickly 5—8 days previous to the next menstruation.

The changes in D seem to vary more or less characteristically. During the actual menstruation or just about at that time D fluctuates very considerably.

During the menstruation cycle the serum reaction undergoes changes, that seem not to have been observed earlier and that seem to be characteristic.

The author believes it most natural to assume that these rhythmical variations in the serum reaction must be attributed to the cyclical changes appearing in the mucous membrane of the uterus during the menstruation cycle. Basing these assumptions on Brdicka's opinion that the increase in polarographic activity in the filtrate

Fig. 45. Blood samples taken during menstrual cycles.

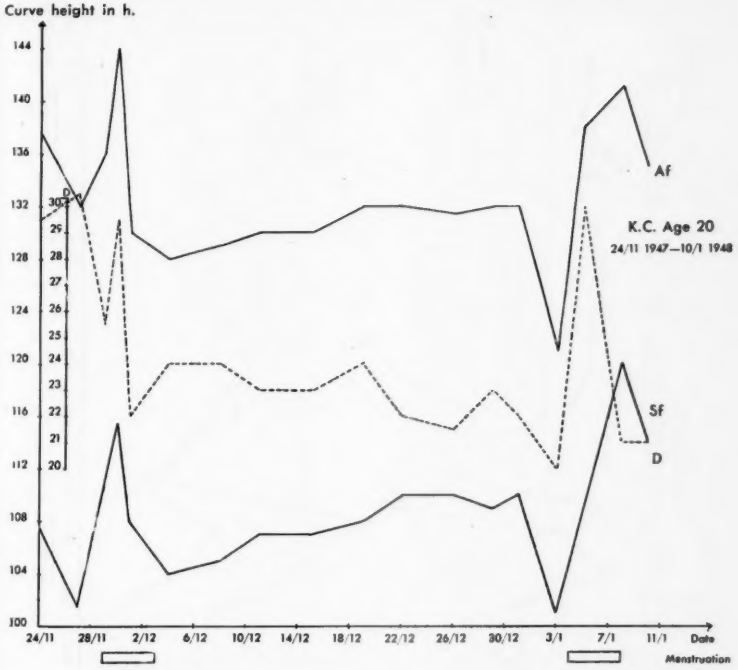

Fig. 46. Blood samples taken during menstrual cycles.

originates from products of decomposition in the blood coming from proteins that have undergone decomposition changes, the variations observed can be explained in quite a simple manner.

The question as to whether pregnancy has any effect on the serum reaction has also received attention. Examinations have been carried out on 8 healthy, pregnant women, and the polarographic values obtained are shown in table 33. From this it will be seen that all of them presented the same values as healthy, non-pregnant women, when tests were taken between 6 and 193 days before partus. In several of the cases the sedimentation rate of the red blood cells was increased—in one case as much as 56 mm/hour, but despite this fact the polarographic filtrate values were perfectly normal.

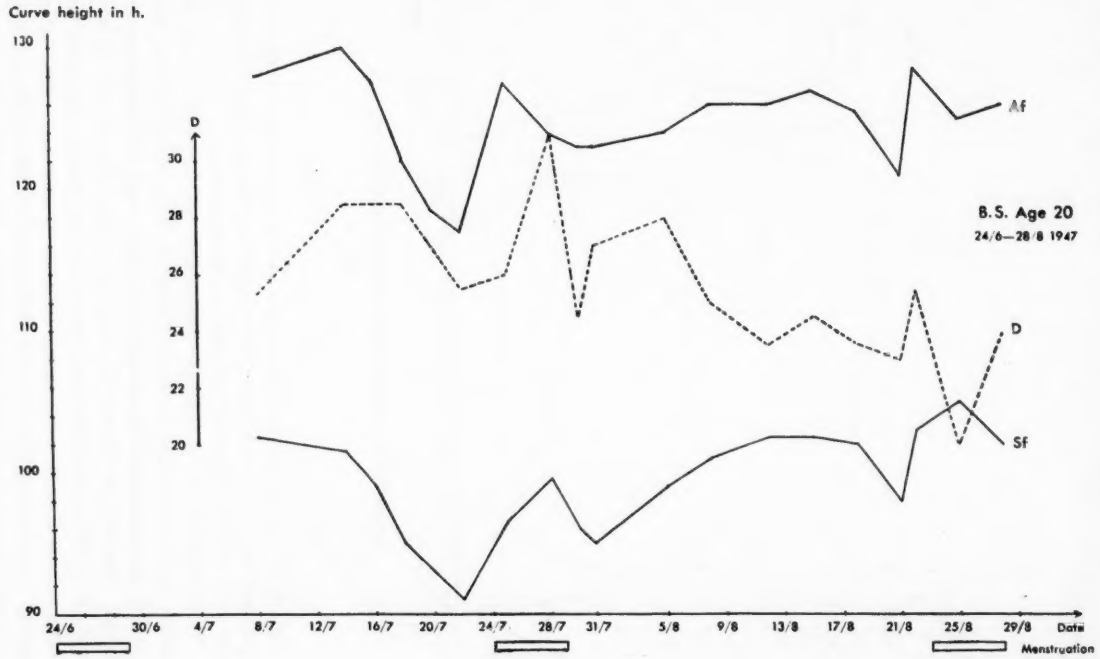

Fig. 47. Blood samples taken during menstrual cycles.

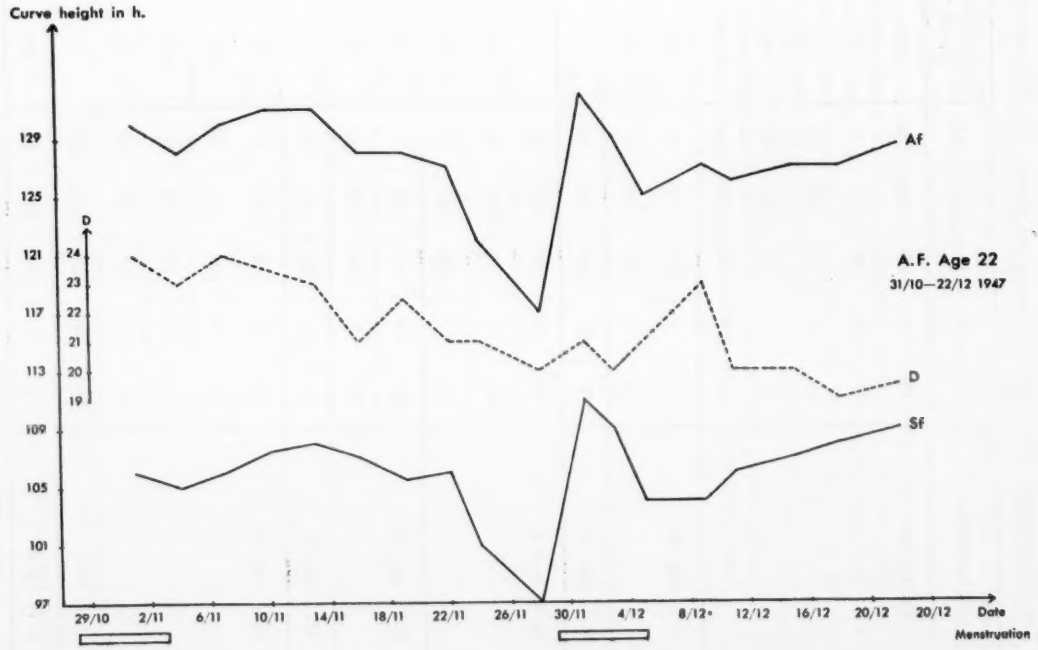

Fig. 48. Blood samples taken during menstrual cycles.

TABLE 33

The polarographic serum reaction during pregnancy. Healthy women.

Case	Hb	SR	Sf	Af	D	Days before partus
S. Ö. Age 25	82	4	108	140	32	193
	84	11	92	122	30	142
	84	10	87	120	33	122
	79	10	92	120	28	80
	83	13	96	117	21	7
S. B. Age 34	78	8	108	139	31	80
	76	6	120	148	28	20
K. L. Age 37	77	56	120	145	25	6
M. B Age 27	74	15	102	126	25	137
	80	14	110	133	23	82
	76	10	108	133	25	14
E. H. Age 26	82	24	103	124	21	45
	79	19	116	139	23	16
M. J. Age 28	88	25	122	145	23	70
E. O. Age 32	86	6	112	139	27	170
	82	9	103	131	28	139
	72	12	98	129	31	102
	76	16	105	128	23	52
	78	14	107	132	25	11
K. L. Age 36	74	18	118	141	23	36

Hb = Haemoglobin values (Zeiss haemometer).
SR = Sedimentation rate of red blood cells in mm/hour according to Westergren's method.

APPENDIX I

It may be suggested that there is a correlation between the protein-content of serum and the polarographic curve-heights. If this is the case, the difference between normal and pathological sera might possibly be caused merely by a difference in protein-content.

In order to study this question, a refractometric determination of the protein-content has been performed on some of the sera used in the present investigation. In all, 93 normal sera, 31 cancer-sera, and 13 sera from infected patients in a febrile state were analysed in this way. The data obtained are shown in fig. 49. Obviously, there is no significant correlation between protein-content and polarographic reaction and the protein-content may thus be considered an unimportant factor for the evaluation of the polarographic data given in the investigation.

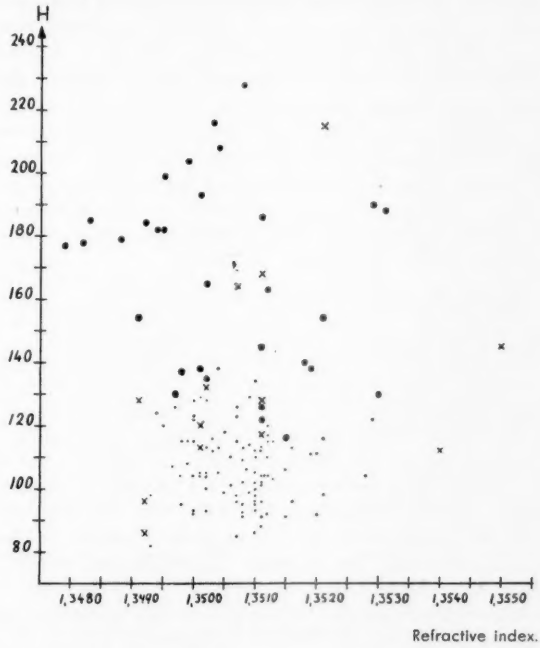

Fig. 49. Refractometric determination of the protein-content and the polarographic curve-heights.
· normal cases. × infectious cases. • cancer cases.

APPENDIX II

Review of recent literature.

Unfortunately, the publication of the present work has been delayed. Thus, the literature collected in the references does not cover the field up to the date of publication. However, an examination of the literature has shown, that the few articles on especially the polarography of serum-proteins do not seriously contradict the present work or render it superfluous. The following review may therefore be made very short.

In some very interesting papers published in 1948 and 1951 (183, 127 a, 185), A. M. Robinson has shown that the polarographic serum-reaction (with alkali denaturation and sulphosalicylic acid precipitation) is "particularly suitable for investigation of prostatic disease." At the same time, it was shown that the administration of oestrogens in this disease causes a decrease in the height of the wave. She recommends the serial examination of sera, with time-intervals of a week to a month, in order to permit differentiation between a benign and a malignant condition. For mastitis and mammary carcinoma, however, the results are reported to be disappointing.

In an extensive study of the "polarographic serum-protein reaction," Forssberg and Nordlander (175) have found that with a rather arbitrary method of evaluation, the method gives i high incidence of erroneous results. Their material is, however, representative of the whole clientèle treated at a cancer clinic, and such a group of patients must be expected to give an appreciable overlapping of values.

Carruthers and Suntzeff (168, 169, 170, 171, 172) have investigated polarographically reducible substances in e.g. normal epidermis and in squamous cell carcinoma by the usual biochemical methods. This has led to the isolation of a water-soluble preparation, exhibiting polarographic activity and with typical ultra-violet extinction-curve. In cancer cases, both these properties changed in a characteristic manner. The small amount of substance available (0.01—0.02 % of the weight of fresh tissue) did not permit an exhaustive chemical analysis, but the data available indicate that the preparation is a complex mixture.

Wintzler and his colleagues (44, 189, 190, 191, 192, 193) have shown that sulphosalicylic acid filtrates of serum contain a non-dialyzable, cystine-containing mucoprotein. Electrophoretic studies of this preparation have shown that it contains at least three components.

Some scattered data on the content of reducing groups in serum, etc., are given in the references (187, 177). For some papers, the lack of experimental data makes a critical review impossible (173, 186). Other papers on similar subjects which will not be reviewed here, but are mentioned for the sake of completeness, are the following (166, 167, 174, 176, 178, 179, 180, 181, 182, 184, 188).

GENERAL DISCUSSION

The "polarographic serum-reaction" has been described above, and some results obtained with it have been reported. On the following pages, the data given will be compared and discussed.

Part I, which deals mainly with the general characteristics of the method and with the results arrived at by other investigators, will only be briefly considered in this discussion.

The most important features of the earlier work are the following. In 1933, the first crude method was invented by Brdicka. In 1938 the same writer published an improvement of his own method. At an early stage it was realized that none of these methods can be considered specific for cancer. Nevertheless, the reaction had a certain relation to the presence of a tumour, to its size, etc., and in subsequent years Brdicka has demonstrated that when critically applied the method is capable of giving useful results.

The question as to the true nature of the substances responsible for the reaction has not yet been settled. It has, however, been claimed that they are mucoproteins, and that they are in some way derived proteins (but they are no longer considered "decomposition-products" of proteins).

In *part II* is given a description of the apparatus, the reagents and the method itself, including the pretreatment of samples, polarographic technique, and evaluation of the polarograms. Attention has been drawn to all critical details, and full instructions are given for all stages of the procedures.

The method may be briefly described as follows. The serum is diluted with water, denatured by heat, precipitated with sulphosalicylic acid or with alcohol, filtered, mixed with a cobalt-containing buffer and polarographed within the voltage-range —0.8 to —1.8 (sulphosalicylic acid) or —0.4 to —1.8 (alcohol). The curve-heights, measured in halves of a millimeter (= the unit h) in the manner described, are called Sf and Af, respectively. The difference Af — Sf is called D.

The error in method amounts to about 0.7 h (table 1, p. 41), which should be compared with the observed ranges of Sf and Af values of about 80—273, respectively. The errors due to the apparatus were found to amount to about 0.55 h (table 2, p. 41). The correct value, however, is certainly lower than this, depending on the fact that the curve-heights decrease slowly with time (cf. e.g. table 7, p. 47). This shows the necessity of accurately fixing the point of time for the performance of the polarographic analysis.

In *part III*, chapters 10 and 11, the method described in part II is used for a methodological study of the polarographic serum-reaction applied to normal and pathological sera. The word "pathological sera"

here means sera from patients with malignant tumours or with an infection in a febrile state.

All cancer cases investigated were more or less advanced and clinically unequivocal. Most of them were confirmed by autopsy or biopsy (for details the casuistry should be consulted). No classification as to degree, malignancy, etc., has been attempted as the object was to study any differences between normal cases, cancer cases and infection cases.

The following purely technical factors were studied:

1) the time between admixture of the precipitant and the carrying out of the filtration (tables 3 and 11),
2) the time required for the mixing of the precipitant with the serum-samples (tables 4 and 12),
3) the time between the transferring of the sample to the electrolysis vessel and the polarographic analysis (tables 7 and 16),
4) the keeping qualities of the filtrates (tables 6 and 15),
5) the keeping qualities of the filtrates after admixture of buffer-solution (tables 5, 13 and 14), and
6) the keeping qualities of the sera (tables 10, 19 and 20).

The results of these tests show that the points 1), 5) and 6) given above are critical, whereas the other three factors may be varied within rather wide limits without harmful effect. Both normal and pathological sera behaved in the same way.

The methodological factors studied are the duration and temperature of the denaturation (only for normal cases and cancer cases). Because of the extensiveness of this part of the investigation, the reader must be referred to the fuller description in the main text (see pp. 48—50, tables 8 and 9 and figs. 13—17, and also pp. 64—66, tables 17 and 18 and figs. 19—32). Here, however, it may be mentioned that

1) the temperature seems to have greater effect than the duration (the greatest change occurring between 50° and 70° C.).
2) the values 12.5 minutes and 80° C. correspond to the centre of the most constant region (fig. 18, p. 55), and
3) normal and cancer sera behave in quite different ways.

The last mentioned fact is especially true for the D-values, which are predominantly positive for normal sera and predominantly negative for cancer sera. A comparison af table 9 and table 18 or of fig. 17 and figs. 30—32 makes this relation clear. Because the difference of the means is of the magnitude 20 h — 30 h, and the error of the method and the error due to the apparatus together amount to only some few h, this difference must be considered highly significant. However, as only very few cases have been studied and no data are available for infectious cases, the difference can not be claimed to prove the difference between cancer sera and other sera.

In *part III*, chapters 12 and 13, an investigation is made of the effect of variations in the alcohol-concentration. This effect is studied for normal, cancer and infectious sera.

The fractionation-curve has been studied in the region 55—75 % alcohol and — for normal sera — with smaller intervals in the region 60.0—64.5 % alcohol. With h-values plotted against % alcohol mean curves are obtained which for all types of sera show three downward bulges (see figs. 33—35, pp. 76—78). However, even more striking is the great difference between on the one hand normal cases with low curves, and on the other the cancer cases and the infectious cases with high curves.

In order to treat this difference in a more quantitative way, due consideration must be paid to the fact that h-values are not directly proportional to the concentrations of reacting SH-groups. On the contrary, the h-values vary with the concentration according to a Langmuir adsorption-isotherm (see p. 11). For this reason, a correction formula has been developed which enables the transformation of h-values to a linear but arbitrary concentration-scale. Calibration data for this correction formula were obtained by diluting known samples in a geometrical progression. Figs. 39—41 (pp. 90—91) show that the difference between the normal and the pathological curves is even more clearly established. A certain difference between the curves for cancer cases and infectious cases is visible, but as this difference consists in a greater variation for the infectious cases, no definite conculsion can be drawn from this observation.

Finally, a comparison is made between the two last steps in the h/%-curves for normal, cancer and infectious cases (figs. 42—44, p. 94). The step-height of fraction no. 2 (f_1) and the fraction no. 3 (f_2) have been measured, and the relative amount of fraction. 2 ($f_1 : f_2$) has been calculated (tables 26 and 27, pp. 96 and 97). It is thus shown that there are statistically significant differences between normal and infectious cases (f_1 and f_2), between normal and cancer cases (also f_1 and f_2) and in one case ($f_2 : f_1$) between cancer and infectious cases.

A glance at the mean curves in figs. 42—44 gives the impression that the f_1-step is much higher for cancer than for normal and infectious cases. The statistical data in table 26, however, show that the difference between cancer cases and infectious cases is not significant. On the other hand, an inspection of table 27 (p. 97) shows clearly that the f_1-values for normal cases and for infectious cases show far fewer variations than the f_1-values for cancer cases. Indeed, the total range is for normal cases 10.5—15.5 and for infectious cases 16.5—25.5, but for cancer cases 21.5—82. Six out of ten values for cancer cases (from 30—82) fall outside the range of the ten infectious cases, and all of them fall outside the range of the ten normal cases. According to the statistical data, the

value 82 corresponds to as much as 16 times the standard deviation for the infectious cases. Because of the small number of cases investigated, this fact is merely pointed out, and no claim is made concerning the possible usefulness of this difference in cancer diagnosis.

The result reported here is a strong indication that useful results may be obtained from much larger experimental series. Unfortunately, it has not yet been possible for the author to perform the work necessary to solve this problem in a fully satisfactory manner. However, the results obtained may also be compared with the results arrived at by Brdicka, who, in an investigation of more than 15,000 cases, has found that the practical value of the so-called "polarographic cancer-reaction" is unquestionable (39). The future will perhaps show that the author's modifications may increase the significance of the method.

The author has not been able to confirm the supposition of Waldschmidt-Leitz and Mayer that in cancer cases a new substance appears in the alcohol-precipitation. On the contrary, what has been observed is that one of the normal steps is increased. (This, of course, does not imply that the substance causing the increase of the step is identical with the normally occurring substance.) It seems obvious that an investigation of serum along the lines described by Carruthers and Suntzeff for epidermis, etc., (see Appendix II) is highly desirable.

In *part IV*, finally, a study is made of the influence of certain physiological conditions on the polarographic values. The reason for this is quite obvious, as it may be suspected that very common physiological states may slightly change the polarographic values. The physiological conditions chosen are such as may often occur in general practice, namely food-intake, physical work, and — for women — menstruation and pregnancy. However, only very few people participated in the tests.

Carbohydrates, fats and proteins were separately tested as food. In all the cases examined no significant changes of the polarographic values have been found.

For the physical work cross-country ski-ing and digging for some hours were used as test-conditions. A reversible increase of Sf and Af values is regularly obtained. This increase is probably dependent on a higher protein-breakdown during prolonged muscular exercise. The D-values show no definite change.

Frequent blood-samples were taken from women during one or two menstruation cycles, and some few samples were taken from pregnant women. During the menstruation cycle, rather large changes of Af and Sf and also of D-values were observed. For a detailed description of the changes the reader must be referred to chapter 16, p. 105, and especially to figs. 45—48.

The few data collected indicate that during pregnancy there is no change of polarographic values for samples taken between 6 and 193 days before partus.

Thus, hard physical work and menstruation are factors which must be considered when evaluating polarographic data according to the polarographic serum-reaction.

Conclusion. Taken together, the data given above indicate that the "polarographic cancer-reaction" of Brdicka should be called the "polarographic serum-reaction". On the other hand, some of the data obtained in this investigation strongly support the idea that there may be significant differences between cancerous and non-cancerous sera. It is also certain, however, that a much more extensive investigation is needed before an unequivocal decision can be made as to the usefulness of this reaction in any respect for cancer-diagnosis.

SUMMARY

The "polarographic serum-reaction" has been studied from methodological points of view and with the aim of finding significant differences between normal sera and sera from pathological cases such as cancer and infectious diseases.

Part I. A survey of the elements of polarography and the "polarographic serum-reaction" is given. After a brief historical treatment the plan of the investigation is presented.

Part II. The apparatus and the reagents used are described. The method and its standardization are treated in detail. The error in method and the error due to the apparatus have been estimated.

The method used is a modification of Brdicka's "polarographic cancer-reaction". The serum has been denatured by heat and then precipitated with sulphosalicylic acid according to Brdicka or with ethyl alcohol according to Waldschmidt-Leitz and Mayer.

Part III. Methodological studies on the various types of sera are described. Some technical factors are shown to be critical. The temperature and the duration of denaturation should be kept in the neighbourhood of the values 80° C. and 12.5 minutes in order to get reproducible results. A variation of the last two values gives different changes for normal sera and for cancer sera.

The alcohol-precipitation has been studied by varying the alcohol-concentration. The results indicate that there are at least three polarographically active components in all filtrates studied. The mean values of the amounts of the two most soluble components are different in normal and in pathological cases. This difference is statistically significant ($P = 0.001$). For the relative proportion of one of the components a significant difference is obtained between cancer-cases and infectious cases. The ranges overlap, however, and it seems too early to assess the value of this finding for the diagnosis of cancer.

Part IV. The polarographic serum-reaction has also been studied under some physiological conditions, including food-intake, rest and work, and menstruation and pregnancy. Food-intake and pregnancy have no influence on the polarograms. After physical work, a reversible increase of the polarographic values is observed. During the menstruation-cycle there were regular and characteristic changes in the polarographic values.

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Table 3.

Case 30.	A.F.	Woman.	Age 22 years.	Healthy.	SR 8 mm/hour.
Case 31.	G.L.	Woman.	Age 26 years.	Healthy.	SR 5 mm/hour.
Case 32.	B.F.	Man.	Age 26 years.	Healthy.	SR 2 mm/hour.
Case 33.	G.S.	Woman.	Age 22 years.	Healthy.	SR 6 mm/hour.
Case 34.	I.W.	Woman.	Age 47 years.	Healthy.	SR 5 mm/hour.

Table 4.

Case 27.	E.Z.	Woman.	Age 32 years.	Healthy.	SR 4 mm/hour.
Case 28.	G.E.	Woman.	Age 17 years.	Healthy.	SR 5 mm/hour.
Case 46.	C.L.	Woman.	Age 22 years.	Healthy.	SR 7 mm/hour.
Case 48.	S.K.	Man.	Age 42 years.	Healthy.	SR 4 mm/hour.
Case 50.	K.H.	Woman.	Age 49 years.	Healthy.	SR 2 mm/hour.

Table 5.

Case	S.Ö.	Man.	Age 33 years.	Healthy.	SR 2 mm/hour.
Case	A.F.	Woman.	Age 19 years.	Healthy.	SR 4 mm/hour.
Case	K.Ö.	Woman.	Age 57 years.	Healthy.	SR 5 mm/hour.

Table 6.

Cases 32, 33, 34 the same as in table 3.

Case 35.	V.K.	Woman.	Age 37 years.	Healthy.	SR 12 mm/hour.

Table 7.

Case S.Ö. and case A.F. the same as in table 5.

Case	T.P.	Man.	Age 35 years.	Healthy.	SR 4 mm/hour.
Case	I.B.	Woman.	Age 19 years.	Healthy.	SR 3 mm/hour.
Case	G.S.	Woman.	Age 25 years.	Healthy.	SR 2 mm/hour.

Table 8.

Case 108.	A.W.	Woman.	Age 48 years.	Healthy.	SR 5 mm/hour.
Case 139.	A.H.	Woman.	Age 55 years.	Healthy.	SR 5 mm/hour.
Case 10.	M.L.	Woman.	Age 39 years.	Healthy.	SR 7 mm/hour.
Case 11.	H.S.	Man.	Age 21 years.	Healthy.	SR 2 mm/hour.
Case 12.	R.A.	Man.	Age 26 years.	Healthy.	SR 4 mm/hour.
Case 13.	S.F.	Woman.	Age 53 years.	Healthy.	SR 10 mm/hour.
Case 14.	N.G.	Woman.	Age 55 years.	Healthy.	SR 4 mm/hour.
Case 15.	L.A.	Woman.	Age 61 years.	Healthy.	SR 6 mm/hour.
Case 16.	S.Ö.	Woman.	Age 35 years.	Healthy.	SR 3 mm/hour.
Case 18.	H.M.	Woman.	Age 49 years.	Healthy.	SR 8 mm/hour.
Case 19.	G.S.	Woman.	Age 23 years.	Healthy.	SR 4 mm/hour.
Case 20.	B.S.	Woman.	Age 22 years.	Healthy.	SR 2 mm/hour.
Case 21.	S.A.	Man.	Age 34 years.	Healthy.	SR 6 mm/hour.
Case 22.	H.L.	Man.	Age 54 years.	Healthy.	SR 4 mm/hour.
Case 23.	M.L.	Woman.	Age 38 years.	Healthy.	SR 8 mm/hour.
Case 24.	F.W.	Woman.	Age 46 years.	Healthy.	SR 12 mm/hour.

Table 9.

Cases 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24 the same as in table 8.

Table 10.

Case	A.H.	Woman.	Age 56 years.	Healthy.	SR	3 mm/hour.
Case	S.Ö.	Man.	Age 34 years.	Healthy.	SR	1 mm/hour.
Case	B.S.	Woman.	Age 18 years.	Healthy.	SR	3 mm/hour.
Case	S.G.	Woman.	Age 42 years.	Healthy.	SR	3 mm/hour.

Table 11.

Case 86. A.W. Man. Age 69 years. Södertälje Hospital. Journal no. 2216/45.
Diagnosis: carcinoma ventriculi.
Mors 28/9. Microscopic diagnosis: stringy small-cells cancer both in the stomach and in the liver, with indications of glandular tissue formation. — SR 8/9: 7 mm/hour. Hemoglobin value: 75 %. Date for blood test: 25/9. Rectal temp.: 37°.2—38°.0 C.

Case 87. G.A.F. Man. Age 56 years. Central county Hospital of Värmland, Karlstad. Journal no. 224/46.
Diagnosis: carcinoma ventriculi.
Laparotomy 19/6: cancer ventriculi inoperabilis. Tumour twice the size of a fist covering the peripheral $^2/_3$ of the stomach both anteriorly and posteriorly. — SR 12/2: 5 mm/hour. Hemoglobin value 8/2: 95 %. Date for blood test: 21/2. Rectal temp.: normal.

Case 88. E.E.L. Woman. Age 54 years. Military Hospital in Boden. Journal no. 2034/45.
Diagnosis: carcinoma ventriculi.
Laparotomy 30/4: carcinoma ventriculi. High up on the anterior wall and minor curvature of the stomach an extensive cancer that has broken through the serosa and extends up towards cardia. The cancer has also penetrated the pancreas, the entire upper part of which is hard and completely fixed. — SR 14/1: 8 mm/hour. Hemoglobin value: 96 %. Date for blood test: 14/1. Rectal temp.: 36°.8—37°.3 C.

Case 89. E.B.B. Man. Age 71 years. Oskarshamn Hospital. Journal no. 1633/45.
Diagnosis: carcinoma recti inoperabilis.
X-ray examination 10/10 1945 showed partial occlusion of the intestinal lumen above ampulla recti. Rectoscopy 16/10: 17 cm above the anus a bleeding tumour. Operation 21/11: carcinoma recti inoperabilis + anus praeternaturalis. Where the colon merges into the rectum a cancer the size of a hen's egg. Near this a metastasis as large as a walnut as well as a couple of smaller ones. — SR 1/10: 46 mm/hour. Hemoglobin value: 63 %. Date for blood test: 14/10. Rectal temp.: normal.

Case 90. E.E.E. Man. Age 66 years. Military Hospital in Boden. Journal no. 1994/45.
Diagnosis: carcinoma pulmonis.
Bronchoscopy 4/1 1946 showed constriction of the right main bronchus from the dorsal side at the branching off of the bronchus of the upper lobe. Microscopic diagnosis: flat-cell cancer. — Hemoglobin value 15/12 1945: 76 %. Date for blood test: 12/1 1946. Rectal temp.: high febrile.

Case 91. O.A. Man. Age 42 years. Nursing Home, Stureby. Journal no. 230/46.
Diagnosis: carcinoma ventriculi (+ metastases).
Mors: 8/8. Pathoanatomical diagnosis: stomach with thick wall, riddled with scirrous cancer. Microscopic diagnosis: tumour of the stomach, but little differentiated, scirrous, strongly infiltrating, growing adenoma. — SR 21/6: 34 mm/hour. Hemoglobin value: 76 %. Date for blood test: 27/6. Rectal temp.: normal-slightly subfebrile.

Case 77. A.S.C. Man. Age 65 years. Central Hospital, Stocksund. Journal no. 1458/45.
Diagnosis: pleuritis exsudativa dext (et abscessus subphenicus?). SR 8/12: 102 mm/hour. Hemoglobin value: 78 %. Date for blood test: 13/12. Rectal temp.: 37°.4—38°.8 C.

Case 78. K.L. Woman. Age 72 years. From the author's own practice.
Diagnosis: pneumonia acuta.
Rectal temp.: 39°.0—40°.1 C.

Case 80. K.K. Man. Age 69 years. Södertälje Hospital. Journal no. 1179/47.
Diagnosis: myodegeneratio cordis c. incompensatio cordis + infarctus embolicus pulmonis ambor. + pleuritis exsudativa dext. SR 7/1: 52 mm/hour. Hemoglobin value 23/12 1946: 87 %. Date for blood test: 7/1 1947. Rectal temp.: 37°.3—38°.2 C.

Case 79. F.N. Woman. Age 55 years. From the author's own practice.
Diagnosis: pneumonia acuta.
Rectal temp.: 38°.5—40°.0 C.

Case 81. M.L. Woman. Age 68 years. Central Hospital, Stocksund. Journal no. 1248/45.
Diagnosis: pleuropneumonia dext (et abscessus pulmonis?).
SR 5/11: 70 mm/hour. Hemoglobin value: 85 %. Date for blood tests: 5/11 and 27/11. Rectal temp.: febrile.

Case 82. J.V.K. Man. Age 70 years. Södersjukhuset. Journal no. 8769/46.
Diagnosis: pneumonia acuta.
SR 27/12 1946: 60 mm/hour. 20/1 1947 35 mm/hour. Hemoglobin value 27/12 1946: 68 %. Date for blood test: 3/1 1947. Rectal temp.: 37°.5—38°.3 C.

Table 12.

Cases 77, 78, 79, 80, 81, 86, 87, 88, 89, 90 the same as in table 11.

Table 13.

Cases 87, 89, 90 the same as in table 11.

Table 14.

Case 211. T.B.H. Man. Age 32 years. Söderby Hospital. Journal no. 4/48.
Diagnosis: tuberculosis pulmonum.
X-ray examination 3/1 1948: right lung riddled with cloudy, exsudative parenchymal indurations. Marked progress since previous control. — SR 31/1: 90 mm/hour. Hemoglobin value 3/1: 80 %. Date for blood test: 3/2. Rectal temp.: 37°.4—38°.1 C.

Case 212. E.P.S. Woman. Age 66 years. Central Hospital, Stocksund. Journal no. 1437/45.
Diagnosis: endocarditis chronica mitralis c. stenosis et insufficientia valvularum + endocarditis chronica aortae + bronchopneumonia + cyanosis organor.
SR 17/12: 81 mm/hour. Hemoglobin value 10/10: 40 %. Date for blood test: 13/12. Rectal temp.: 37°.4—38°.8 C.

Case 213. B.T.R. Woman. Age 29 years. Söderby Hospital. Journal no. 838/47.
Diagnosis: tuberculosis pulmonum.
X-ray examination 10/11 1947: In the upper part of the lung-parenchym of the right lung a conglomerate of cavities ranging in size up to that of an orange. The cavities surrounded with massive indurations. SR 7/2 1948: 105 mm/hour. Hemoglobin value 8/11 1947: 75 %. Date for blood test: 3/2 1948. Rectal temp.: 36°.8—37°.5 C.

Table 15.

Cases 87, 89, 90, 82 the same as in table 11. Case 214 the same as no. 213 in table 11.

Table 16.

Cases 77, 78, 79, 87, 89, 90 the same as in table 11.

Table 17.

Case 221. O.A.A. Man. Age 42 years. Nursing Home, Stureby.
Diagnosis: carcinoma ventriculi inoperabilis (with metastasis in sternum).
SR 21/6 1945: 34 mm/hour. Hemoglobin value: 76 %. Date for blood test: repeated tests during June 1945. Rectal temp.: normal.

Case 224. The same as no. 91 in table 11 (but a new blood test).

Case 225. A.K.P. Woman. Age 65 years. Nursing Home, Stureby.
Diagnosis: carcinoma ventriculi inoperabilis et ascites.
Laparotomy autumn 1945: large cancer metastases in abdomen. — SR 29/8 1946: 146 mm/hour. Hemoglobin value: 40 %. Date for blood test: repeated tests during August 1946.

Case 230. J.S. Man. Age 59 years. Central Hospital, Örebro. Journal no. 2471/46.
Diagnosis: carcinoma pulmonis.
X-ray examination 8/10 1946: massive induration of the right side of thorax caused by an exsudate. Mors 22/10. Pathoanatomical diagnosis: cancer bronchiale in the inferior lobe of the left lung. Multiple cancer metastases. Probably primary lung cancer. — Date for blood test: 8/10. Rectal temp.: 37°.4—38°.0 C.

Case 231. J.I.V.L. Man. Age 51 years. Central County Hospital of Värmland, Karlstad. Journal no. 1182/45.
Diagnosis: carcinoma ventriculi inoperabilis.
Laparotomy 20/10: completely inoperable cancer of the stomach. — SR 10/10: 42 mm/hour. Hemoglobin value: 53 %. Date for blood test: 10/10. Rectal temp.: 36°.4—40°.7 C. (febris intermittens).

Case 232. E.M.R. Woman. Age 59 years. Sabbatsberg Hospital, Stockholm. Journal no. M II 251/46.
Diagnosis: carcinoma coli (+ metastases).
Mors 22/7. Pathoanatomical diagnosis: in the lower part of the colon a cancer with central, rather slight ulceration. Carcinoma coli with metastases in the lymph-nodes, liver, lungs, peritoneum and pleuræ + hydrothorax haemorrhagicus sin. + ascites + embolia in the left ramification of the minor lung artery + thrombosis in the femoral veins. — SR 11/6: 40 mm/hour. Hemoglobin value 5/6: 62 %. Date for blood test: 7/6. Rectal temp.: 37°.3—38°.0 C.

Case 233. E.A.C. Man. Age 70 years. Sabbatsberg Hospital, Stockholm. Journal no. K II 1178/46.
Diagnosis: carcinoma ventriculi (+ metastases in the lymph-nodes).
Laparotomy 16/7: an enormous tumour, probably proceeding from the minor side or the posterior wall of the stomach and occupying a large part of that organ. — Microscopic diagnosis: adenocarcinoma of gelatinous type with rather polymorphous nuclei, rather abundant connective tissue. — SR 13/7: 76 mm/hour. Hemoglobin value 15/7: 70 %. Date for blood test: 16/7. Rectal temp.: 37°.0—38°.5 C.

Case 234. F.N.D. Man. Age 53 years. Södertälje Hospital. Journal no. 768/46.
Diagnosis: carcinoma ventriculi.
Exploratory laparotomy in Nov. 1946: upper part of the abdomen filled with tumourus masses enveloping the stomach and the pancreas. — Mors 7/11. Pathoanatomical diagnosis: cancer ventriculi the size of a fist proceeding from the upper part of curvatura minor. The tumour adherent posteriorly to the spine. Metastases of the liver. — SR 21/10: 52 mm/hour. Hemoglobin value 6/10: 72 %. Date for blood test: 22/10. Rectal temp.: 37°.0—37°.7 C.

Case 236. Man. Age 63 years. Åsö Hospital.
Diagnosis: carcinoma pulmonis.
1934 lobectomi. 1945 explorative laparotomy: cancer metastases in the abdomen. Date for blood test: 30/8 1946.

Case 245. E.M.A. Woman. Age 43 years. Södertälje Hospital. Journal no. 192/46.
Diagnosis: carcinoma coli (+ metastases).
Laparotomy 20/2: cancer coli the size of a plum at flexura hepatica and, behind this, several hard lumps ranging up to the size of a pigeon's egg in the mesocolon and the posterior abdominal wall (lymph node metastases). — Mors 13/3. Pathoanatomical diagnosis: adenocarcinoma coli. — SR 11/2: 3 mm/hour. Hemoglobin value: 87 %. Date for blood test: 19/2. Rectal temp.: 37°.3—38°.8 C.

Case 246. S.J.A. Woman. Age 72 years. Södertälje Hospital. Journal no. 760/46.
Diagnosis: carcinoma coli transversi.
Laparotomy 13/9: cancer of the colon. Microscopic diagnosis: adenocarcinoma coli. — SR 28/8: 40 mm/hour. Hemoglobin value 21/8: 70 %. Date for blood test: 28/8. Rectal temp.: 37°.4—38°.4 C.

Table 18.

The same cases as in table 17.

Table 19.

Cases 87, 88, 89, 90 the same as in table 11. Case 113 the same as 232 in table 17.

Table 20.

Case 730 the same as 211 in table 14.

Case 731. A.B.J. Man. Age 25 years. Söderby Hospital. Journal no. 738/47.
Diagnosis: tuberculosis pulmonis.
X-ray examination 4/12 1947: changes in the right lung of exsudative tuberculous character with liquefaction. — SR 29/1 1948: 32 mm/hour. Hemoglobin value 9/12 1947: 92 %. Date for blood test: 29/1 1948. Rectal temp.: 36°.9—37°.7 C.

Case 732. M.K.R. Woman. Age 33 years. Söderby Hospital. Journal no. 819/47.
Diagnosis: tuberculosis pulmonis.
X-ray examination 21/1 1947: blurred and slightly saturated patches on the right lung as also on the left. In the left lung also a system of cavities. — SR 22/1 1948: 28 mm/hour. Hemoglobin value 4/11 1947: 90 %. Date for blood test: 30/1 1948. Rectal temp.: afebrile.

Case 733. K.L. Woman. Age 49 years. From the author's own practice.
Diagnosis: pneumonia acuta. Rectal temp.: 39°.8 C.

Case 734 the same as case 81 in table 11.

Table 21.

Case 1. The same as "A.F." in table 3.
Case 2. The same as "G.L." in table 3.
Case 3. The same as "B.F." in table 3.
Case 4. The same as "I.W." in table 3.
Case 5. The same as "E.Z." in table 4.
Case 6. The same as "S.K." in table 4.
Case 7. The same as "K.H." in table 4.
Case 8. The same as "B.F." in table 3.
Case 9. The same as "K.Ö." in table 5.
Case 10. The same as "S.G." in table 5.

Table 22.

Case 1. The same as case 221 in table 17.

Case 2. S.H.R. Woman. Age 55 years. Nursing Home, Stureby.
Diagnosis: carcinoma recti.
Laparotomy 1944: cancer recti. Anus praeternaturalis. — SR 23/5 1946: 7 mm/hour. Hemoglobin value: 81 %. Date for blood test: 28/6 1946. Rectal temp.: normal.

Case 3. N.I.F. Man. Age 76 years. Nursing Home, Stureby. Journal no. 70/46.
Diagnosis: carcinoma coli.
X-ray examination 15/8 1946: occlusion of lumen in colon transversum over length of 10 cm. Here possible to palpate a mobile tumour. Laparotomy 27/8: cancer coli. — Mors 26/11. — SR 6/8: 64 mm/hour. Hemoglobin value: 83 %. Date for blood test: 21/8. Rectal temp.: about 38° C.

Case 4. The same as case 233 in table 17.

Case 5. C.G.S. Man. Age 72 years. Sabbatsberg Hospital, Stockholm. Journal no. M II 423/46.
Diagnosis: carcinoma ventriculi.
Laparotomy 18/7: a cancer of the stomach the size of a fist in the posterior wall of the stomach and extending to pancreas, spleen and posterior abdominal wall. Not accessible for biopsy.

Case 6. N.E.E. Man. Age 62 years. Nursing Home, Stureby.
Diagnosis: carcinoma bronchiale.
Microscopic diagnosis: flat-cell cancer. — SR 22/7: 32 mm/hour. Hemoglobin value: 34 %. Date for blood test: 21/8 1946.

Case 7. J.L. Man. Age 80 years. Nursing Home, Stureby.
Diagnosis: carcinoma prostatae (+ metastases in the vertebræ). SR 17/8: 67 mm/hour. Hemoglobin value: 65 %. Date for blood test: 25/8 1946.

Case 8. E.M.N. Woman. Age 57 years. Nursing Home, Stureby.
Diagnosis: carcinoma uteri.
Patient treated with radium 1945. 24/8 1946: large tumorous masses filling both rectum and vagina. — SR 24/8: 43 mm/hour. Hemoglobin value: 60 %. Date for blood test: 25/8 1946.

Case 9. The same as case 236 in table 17.

Case 10. The same as case 225 in table 17.

Table 23.

Case 1. The same as case 77 in table 11.

Case 2. The same as case 213 in table 14.

Case 3. The same as case 81 in table 11.

Case 4. The same as case 80 in table 11.

Case 5. G.M.J. Woman. Age 26 years. Central Hospital, Stocksund. Journal no. 1268/47.
Diagnosis: pleuritis exsudativa.
X-ray examination 8/11: massive induration on the left side up to the level of the 4th rib. Above this induration, laterally in the parenchyma a massive induration the size of a child's palm, probably decapsulated exsudate. — SR 17/11: 70 mm/hour. Hemoglobin value 1/12: 80 %. Date for blood test: 18/11. Rectal temp.: 38°.0—40°.1 C.

Case 6. The same as case 211 in table 14.

Case 7. C.H. Woman. Age 52 years. From the author's own practice.
Diagnosis: pneumonia acuta.
Rectal temp.: febrile.

Case 8. The same as case 79 in table 11.

Case 9. The same as case 733 in table 20.

Case 10. M.B.I.B. Woman. Age 26 years. Söderby Hospital. Journal no. 751/47.
Diagnosis: tuberculosis pulmonis.
SR 22/1 1948: 12 mm/hour. Hemoglobin value 14/1: 90 %. Date for blood test: 30/1. Rectal temp.: 36°.7—37°.2 C.

Table 24.

Case 432. A.F. The same case as in table 3.
Case 433. S.Ö. The same case as in table 5.
Case 444. K.Ö. The same case as in table 5.
Case 445. B.F. The same case as in table 3.
Case 446. C.L. The same case as in table 4.
Case 447. A.H. The same case as in table 8.
Case 448. H.S. The same case as in table 8.
Case 449. R.A. The same case as in table 8.
Case 450. H.M. The same case as in table 8.
Case 451. G.S. The same case as in table 8.
Case 452. S.A. The same case as in table 8.
Case 453. H.L. The same case as in table 8.
Case 454. M.L. The same case as in table 8.
Case 455. S.G. The same case as in table 10.

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